Blackberries (Rubus ulmifolius Schott) from Calabria (Italy): a comprehensive characterisation.

Blackberries (*Rubus ulmifolius* Schott) from Calabria (Italy): a comprehensive characterisation

ArticleCategory : Original Paper

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Rec: 9 September 2021, Rev: 12 November 2021, Accp: 20 November 2021,

Abstract

The establishment of the corresponding standards of quality to promote the commercialization of *Rubus ulmifolius* Schott (Rosaceae) blackberries from Calabria (Italy) has been aimed in this study. Data on the volatile composition gathered by Solid-Phase Microextraction (SPME) followed by GC–MS have been complemented with the study for the first time of physicochemical parameters and antioxidant capacity of wild and cultivated blackberries collected in seven different locations of Calabria. Wild fruits showed significantly higher dry matter content (16.34–22.14%) and pH (3.92–5.12) and lower total acidity (0.23–0.74 as % of citric acid) compared to the cultivated sample (dry matter: 15.31%; pH: 3.30 and total acidity: 1% citric acid), whereas colour and total soluble solids were similar. Antioxidant capacity (DPPH assay) of wild blackberries, correlated ($r = 0.71$) with results from ABTS assay, was significantly higher ($SE_{50} = 1.6–3.4$ mg DW), in agreement with its higher content of total anthocyanins and total phenolics. Ethanol (11.8–32.4%), *trans*-2-hexenal (2.7–21.3%), methylbutanal (5.7–17.4%), and ethyl acetate (4.6–11.9%) were the major compounds in both wild and cultivated blackberries. Although the presence or relative concentration of several volatiles (e.g. *p*-cymen-8-ol, decanal, 3-hydroxy esters, etc.) seemed to be characteristic of the harvest location/blackberry type, further research on a higher number of samples should be carried out to confirm these results. The comprehensive characterisation addressed for the first time in this paper is a valuable preliminary contribution to satisfy the demand by consumers and farmers of objective data to support the premium quality of Calabrian blackberries.

Keywords: Blackberries (*Rubus ulmifolius* Schott), Calabria (Italy), Physicochemical data, Antioxidant activity, Volatiles, Food characterisation

Introduction

Blackberry (*Rubus* sp.) fruits have long been collected and consumed, not only for their pleasant aroma and taste [1–5], but also for their high nutritional value and bioactive properties associated with the wide variety of phytochemicals (vitamins, minerals, polyphenols, etc.) they possess [6–9]. Thus, among other health benefits, blackberries have been reported to have antioxidant, anti-carcinogenic, anti-inflammatory, antimicrobial, anti-diabetic, anti-diarrheal, and antiviral activities [10–12]. All these properties have contributed not only to their increasing popularity as part of a healthy human diet [13, 14], but also to enlarge their economic importance in the food industry as a source of natural flavourings or food pigments, as raw material for the elaboration of different foodstuffs (liquors, juices, jams, syrups, pastries, etc.), etc.

Blackberry aroma, directly related to its volatile composition, is also decisive as regard as the appreciation of this berry by consumers. As for bioactives, blackberry volatile profiles and odour-active compounds are affected by many factors such as the genotype, the pre-harvest and post-harvest conditions, etc. As an

example, 'Marion' blackberries characterised by fresh fruit and strawberry notes, show high contents of acids and esters, and are preferred by some consumers over 'Thornless Evergreen' blackberries, with higher alcohol content, and a vegetal and woody character [3]. Although different methodologies have been described in the literature for volatile profiling of berries belonging to the *Rubus* genus, methods based on the use of Solid-Phase Microextraction (SPME) followed by GC–MS have scarcely been applied so far for the study of the aroma of blackberries, despite their advantages in terms of speed, simplicity, affordability, sensitivity, etc. [5, 15–18].

Rubus sp. blackberries have been described to be a good source of natural antioxidants with a remarkably high scavenging activity towards chemically generated superoxide radicals [14, 19–22]. This bioactivity, attributed to a variety of constituents but mainly to phenolic compounds, has been evaluated by a large number of in vitro tests [23, 24]. As one of the main classes of phenolics in blackberries, the results of many studies have evidenced that the antioxidant activity of this fruit is mainly correlated with its anthocyanin content [25, 26]. Other health-promoting activities associated with the phenolic composition of blackberries include anti-inflammatory [14, 27], anticancer [8, 14], etc.

Rubus ulmifolius Schott is an evergreen shrub of 0.25–2 m high, having prickly stems, compound leaves consisting of 3–5 leaflets, white or pink flowers (2–3 cm) in pyramidal inflorescence and black (when ripened) fruits with excellent organoleptic properties. *R. ulmifolius* Schott is widespread all over Italy up to 1100 m above the sea level, and it is one of the most frequent species of bramble, often invasive in urban and suburban ecotypes [28, 29]. Despite both the climatological and soil conditions of Calabria (Southern Italy) make the spontaneous growth of blackberries favorable, harvesting of wild blackberries is mainly considered as an entertainment activity and only a relatively extensive cultivation area, as compared to other typical Calabrian crops, is dedicated to blackberry production. Cultivation of varieties (adapted or not from this wild species) could be, therefore, considered as a profitable resource for economic revalorisation of this region. To this aim, studies that comprehensively evaluate both the changes in aromatic composition and bioactivity with harvest year are highly demanded, as they contribute not only to the characterisation of these samples, but also to further establish their standards of quality.

A single report by D'Agostino et al. [17] on optimization of a SPME method for isolation of volatiles prior to their gas chromatographic-mass spectrometric analysis has been previously applied to the characterisation of the volatile composition of *R. ulmifolius* Schott blackberries collected in 2012 in different locations of Calabria (Italy) and Spain. As novelty over this reference, a comprehensive characterisation by SPME GC–MS of the volatile composition of wild and cultivated *R. ulmifolius* Schott blackberries collected in the same locations of Calabria but at a different harvest year aimed to evaluate the stability of the aromatic profile with the harvesting conditions, has been complemented with the study for the first time in this paper of their physicochemical parameters and antioxidant activity. Results from this research are a valuable contribution in different fields such as food science, agriculture, etc., as aroma and antioxidant activity are two of the main attributes of blackberries valued by consumers.

Materials and methods

Chemicals

Methanol and anhydrous sodium carbonate were purchased from Carlo Erba Reagents (Milan, Italy). Potassium chloride, sodium acetate, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 0.1 N sodium hydroxide, gallic acid and the 2 N Folin-Ciocalteu reagent were obtained from Sigma-Aldrich Co. (Milan, Italy). Purity of all chemicals, except for DPPH (> 90%), was higher than 97.5%. All other reagents were from Sigma-Aldrich Co. (Milan, Italy).

Samples

A total of seven 500 g-blackberry (*Rubus ulmifolius* Schott) samples harvested in June 2011 at their full-ripe stage were subjected to analysis: six wild blackberry samples were harvested at different locations in Calabria (Southern Italy), and one cultivated *R. ulmifolius* Schott ('Loch Ness' cultivar) sample was obtained from a greenhouse in Bovalino (Calabria). Sample codes and harvest locations/altitudes are listed in Table 1.

Table 1 Calabrian wild and cultivated *Rubus ulmifolius* Schott blackberries under analysis

Graphical abstract

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Sample code	Location	Altitude (m)	Latitude	Longitude
COS	Cosoleto	429	38° 16′33″ N	15° 55′43″ E
FIL	Filadelfia	505	38° 46′36″ N	16° 17′25″ E
ROS	Rosarno	66	38° 29′08″N	15° 58′47″ E
CIC	Cicerna	27	38° 27′34″ N	15° 55′25″ E
GRA	Granatara	12	38° 29′14″ N	15° 57′04″ E
NIC	Nicotera	7	38° 32′03″ N	15°56′23″ E
BOV	Bovalino	11	38° 14′15″ N	16°15′59″ E

Portions of each of these seven blackberry samples were differently processed according to the scheduled type of analysis. Fresh portions were immediately used for physicochemical assays, while the frozen ones (stored in the dark at − 20 °C for less than 2 weeks) were subsequently employed for antioxidant analysis (DPPH). Finally, freeze-dried blackberries aimed to ABTS assay and volatile determination were processed as whole berries using a Lyoalfa 6 freeze-drier (Telstar, Italy), and were stored at − 20 °C for better preservation until analysis.

As for physicochemical characterization and antioxidant analysis, three randomized batches (10 g each) for every harvesting location were considered for analysis, whereas freeze-dried batches for the same collection place were combined, powdered and sieved (< 0.5 mm) before volatile profiling.

Physicochemical characterization

Colour was measured at 25 °C using a Konica Minolta CM-700d/600d spectrophotometer (Konica Minolta Sensing, Inc., Japan). Data were expressed as L* (lightness/darkness in the range 0–100), a* (greenness/redness in the range between − 60 and + 60) and b* (blueness/yellowness in the range between − 60 and + 60) coordinates.

A Crison basic 20 pH meter (Crison strumenti SpA, Modena, Italy) was used to measure the pH of blackberry homogenates obtained from 10 g of blackberry pulp and 90 mL of deionised water ~~by~~ using an Ultra-Turrax T-25 homogeniser (IKA Labortechnik, Janke & Kunkel, Saufen, Germany) operating at 24,000 rpm for 1 min. This solution was then titrated with 0.1 N NaOH to pH 8.1, according to Du et al. [3]. Titratable acidity (TA) was expressed as citric acid percentage on a fresh weight (FW) basis.

Soluble solid content (SSC) was measured at 20 °C using a digital Atago Model PR-101α refractometer (Atago Co. Ltd, Milan, Italy). Results were reported as Brix grades (ºBx). Dry matter content (DMC, %) was gravimetrically determined by drying 10 g of homogenised fresh samples in a PID System oven (Artiglass SRL, Padua, Italy) at 105 °C until constant mass.

All determinations above described were made in triplicate for each of the three sample batches considered per harvesting location.

Volatile analysis

Solid-phase microextraction

Isolation of volatiles by SPME was done according to the method previously optimised by D'Agostino et al. [17]: 0.2 g of freeze-dried blackberries were weighed into a 5-mL glass vial sealed by means of a screw cap provided with a predrilled Teflon-faced septum (Supelco, Bellefonte, PA). Volatiles were sampled using an 85 µm Carboxen™-Polydimethylsiloxane StableFlex (Supelco) fiber at an extraction temperature of 66 °C, following an incubation time of 20 min and an extraction time of 16 min.

Gas ~~chromatography-mass~~ chromatography–mass spectrometry (GC–MS) analysis

GC–MS analyses (n = 3) were performed on an Agilent 6890 (Palo Alto, CA, USA) gas chromatograph coupled to a Hewlett-Packard 5973 quadrupole mass detector. The SPME fiber was desorbed into the injection port at 250 °C in splitless mode (3 min) using a SPME liner (78.5 mm length × 6.5 mm external diameter × 0.75 mm internal diameter, Supelco). Separation was achieved on a Supelcowax column (27.2 m length × 0.25 mm internal diameter × 0.25 µm film thickness, Supelco), using helium as carrier gas (0.8 mL min^{-1}). The oven temperature was raised from 40 °C (3 min) to 220 °C at 3 °C min^{-1}. Mass spectra were recorded in electron impact (EI) mode at 70 eV, scanning the 35–450 m/z range. Interface and ionisation source temperatures were 280 and 230 °C, respectively.

Identification of volatile compounds was carried out by comparison of their experimental mass spectral fragmentation patterns with those of standards in the Wiley mass spectral library [30]. Linear retention indices (I^T) experimentally obtained and data from the literature were used for further confirmation of identifications. Semiquantitative data for every volatile were calculated as percentage of total volatile composition determined.

Antioxidant analysis

Extraction procedure

Samples of unfrozen blackberries (10 g-batch) were homogenised (1 min at 24,000 rpm) with 30 mL of a methanol/water/hydrochloric acid (80:19.9:0.1, % v/v) solution using an Ultra-Turrax T-25 homogeniser (IKA Labortechnik), and further extracted at room temperature for 1 h in the dark under continuous stirring. The residue obtained by vacuum-filtration (Whatman no. 1 filter, Vetrotecnica SRL, Padua, Italy) was re-extracted three times (until colourless) under the same conditions to maximise the antioxidant recovery. The filtrates were combined and evaporated to dryness using a rotary evaporator R-200 (Büchi, Italy) operating at 40 °C. Then, stock solutions were prepared for further antioxidant analysis using 30% MeOH as solvent. The whole extraction procedure was repeated for the three batches per sample previously described.

Total anthocyanin content (TAC)

Total monomeric anthocyanins were determined by the pH differential method [31]. Briefly, this method is based on the reversible structural change of the anthocyanin chromophore at pH 1.0 (highly coloured) and at pH 4.5 (colourless). Blackberry stock solutions were diluted 1:5 with pH 1.0 (0.025 M potassium chloride) or pH 4.5 (0.4 M sodium acetate) solutions. After equilibration at room temperature for 15 min, the absorbance was measured (n = 3) at 520 nm (maximum absorbance of cyanidin 3-glucoside, Cyd-3-Glu) and at 700 nm (for turbidity corrections) on an Agilent 8453 UV/Vis Spectrophotometer G1103A with 89090A Peltier Temperature Control (Agilent Technologies, Turin, Italy). Results were expressed as mg of Cyd-3-Glu equivalents g^{-1} dry weight (DW).

Total phenolic content (TPC)

TPC was determined by the Folin-Ciocalteu colorimetric method, modified as in Tomaino et al. [32]. Briefly, 50 µL of stock solution was shaken for 3 min with 450 µL of distilled water and 500 µL of 2 M Folin–Ciocalteu reagent. Subsequently, 500 µL of a 10% (w/v) sodium carbonate solution were added. Solutions were mixed and allowed to stand at room temperature in the dark for 1 h. After centrifugation (16467 g, 3 min), the absorbance at 786 nm was measured (n = 3) by using an Agilent 8453 UV/Vis Spectrophotometer G1103A with 89090A Peltier Temperature Control. The same procedure was repeated with hydro-organic solutions of gallic acid in the 1.25–20 µg mL^{-1} concentration range to build up the corresponding calibration curve. Results were expressed as mg of gallic acid equivalents (GAE) g^{-1} DW.

Antioxidant capacity (AC) by DPPH and ABTS assays

Two different in vitro assays were used to evaluate the antioxidant capacity (AC) of the samples under study. Determination of the free radical scavenging capacity against DPPH was done as described in a study on *Rubus idaeus* L. [33], with slight modifications. In brief, 37.5 µL of stock solutions diluted in 30% MeOH were added to 1.5 mL of DPPH solution (0.025 g L^{-1} in methanol). The mixtures were shaken vigorously and left stand at room temperature in the dark for 20 min. The decrease in the absorbance after 20 min was determined (n = 3) at 515 nm using an Agilent 8453 UV/Vis Spectrophotometer G1103A provided with 89090A Peltier Temperature Control. Results were expressed as mg of DW required to scavenge 50 µmol of

initial DPPH concentration in the reaction mixture (SE_{50}).

The ABTS (2,20-azinobis-3-ethylbenzothiazoline-6-sulfonate) radical assay was carried out as proposed by Re et al. [34]. An Agilent 8453 UV/Vis Spectrophotometer G1103A provided with 89090A Peltier Temperature Control was used. Briefly, a solution of ABTS radical was diluted (1:80) with ethanol to give an absorbance of 0.70 at $k = 734$ nm. An aliquot of extract was added to ABTS solution. Trolox was used as a standard antioxidant, and fruit activity was expressed in μmol of Trolox equivalents g^{-1} DW.

Statistical analysis

Data were subjected to one-way analysis of variance (Duncan test) using the SPSS software v. 17.0.0 for Windows (SPSS Inc, 2006). The significance of differences was defined as $P < 0.05$. Multiple regression analysis of antioxidant data was carried out using the Statistica software v. 7.1 (Statsoft, 2005).

Results and discussion

Physicochemical characterisation

In general, a great homogeneity was found for physicochemical data in all the wild samples under study, irrespective of their harvest location (Table 2). For the colour assay, no significant differences between wild and cultivated samples were determined. Similar average Hunter CIE-Lab parameters have also been reported for the nine genotypes of ripe wild blackberries (*Rubus* L.) from Samsun (Turkey) analysed by Tosun et al. [35]. Moreover, and in agreement with data published by Patras et al. [36], data here reported for Hunter a* value matched well with values determined for unprocessed blackberry purées and were noticeably higher than those described for samples subjected to either thermal or high pressure processing. Therefore, and although further studies (e.g. analysis of carbohydrate composition) would be required to confirm this hypothesis, colour results listed in Table 2 seem to point at a similar ripening stage for all blackberries analysed in this study.

Table 2 Physicochemical data (mean ± standard deviation, $n = 3$) of Calabrian *R. ulmifolius* Schott blackberries

Samples	DMC (%)	pH	TA (% citric acid)	SSC (°Bx)	Colour		
					*L**	*a**	b*
COS	21.43 ± 2.58^{a*}	4.08 ± 0.47^{b}	0.47 ± 0.05^{b}	13.77 ± 1.90^{b}	27.61 ± 1.35^{a}	0.96 ± 0.42^{a}	− 1.27 ± 0.24^{b}
FIL	21.85 ± 0.80^{a}	4.64 ± 0.29^{ab}	0.30 ± 0.05^{a}	15.03 ± 0.51^{ab}	32.71 ± 5.44^{a}	0.99 ± 0.86^{a}	− 0.78 ± 0.47^{ab}
ROS	20.24 ± 0.62^{a}	5.12 ± 0.67^{a}	0.23 ± 0.04^{a}	13.57 ± 1.10^{b}	30.92 ± 6.44^{a}	0.59 ± 0.14^{a}	− 1.11 ± 0.35^{b}
CIC	22.14 ± 2.70^{a}	4.24 ± 0.68^{b}	0.26 ± 0.04^{a}	19.60 ± 4.69^{a}	27.33 ± 0.92^{a}	1.07 ± 0.80^{a}	− 0.71 ± 0.44^{ab}
GRA	16.34 ± 0.76^{b}	3.92 ± 0.59^{bc}	0.74 ± 0.08^{c}	11.43 ± 2.47^{b}	29.74 ± 4.61^{a}	2.55 ± 1.04^{a}	1.78 ± 3.32^{a}
NIC	21.26 ± 0.95^{a}	4.61 ± 0.12^{ab}	0.33 ± 0.07^{bc}	12.93 ± 1.69^{b}	30.13 ± 3.29^{a}	5.34 ± 5.66^{a}	0.37 ± 1.16^{ab}
BOV	15.31 ± 0.72^{b}	3.30 ± 0.04^{c}	1.00 ± 0.19^{d}	14.97 ± 3.76^{ab}	32.86 ± 5.69^{a}	3.93 ± 2.68^{a}	0.41 ± 0.44^{ab}

*Entries for each physicochemical parameter followed by the same letter showed no statistically significant differences for their mean value at the 95% confidence level

Regarding DMC, no significant differences at the 95% confidence level were found among wild blackberries (COS, FIL, ROS, CIC, NIC); wild sample GRA and cultivated sample BOV showed similar and lower results (Table 2). DMC of most of Calabrian *R. ulmifolius* Schott blackberries here analysed was in the range previously reported for 'Marion' and 'Evergreen' blackberries (18–24%) [24] and for other *Rubus* blackberries such as *R. glaucus* Benth. ('Andean blackberry') and *R. adenotrichus* (16.5–18.5%) [19].

pH assay was in the range 3.3–5.1; the lowest value was determined for samples GRA and BOV (pH = 3.9 and 3.3, respectively) and the highest (pH = 5.1) for sample ROS (Table 2). These results are in good

agreement with those previously described for 'Marion' (pH = 3.13–3.16) and 'Evergreen' (pH = 4.28–4.40) blackberries [24, 37, 38]. However, data experimentally determined in this study are slightly higher than those reported for different blackberry cultivars grown in Slovenia and sampled at optimal ripening stage (pH = 2.84–3.11) [39] and those of *R. adenotrichus* blackberries from Costa Rica (pH = 2.83) and 'Andean blackberries' from Ecuador (pH = 2.98) [19].

Titratable acidity was significantly higher for samples GRA and BOV (TA > 0.74% citric acid), as compared to the remaining wild blackberries under study. A wide variability in TA has been described to be associated with the blackberry genotype/cultivar considered (data in the range 0.7–3% citric acid) [3, 40], the ripeness stage [37, 39] and the processing and storage conditions [24, 40], among other factors.

Except for wild samples FIL and CIC and cultivated BOV sample, total soluble solids measured for Calabrian blackberries were in the range (9–14 ºBx) generally described for different *Rubus* blackberry species and genotypes [3, 13, 19, 20, 41, 42]. Higher levels of SSC, similar to those of FIL, CIC and BOV samples, have also been described for 'Marion' and 'Evergreen' blackberries from USA [24, 37].

Volatile analysis

Although the analysis of the volatile composition of different *Rubus* blackberries, specially *Rubus laciniata* L. and *Rubus glaucus* Benth. [1–5, 15, 43], has been the aim of a number of studies, little attention has been paid to the evaluation of the aroma of *Rubus ulmifolius* Schott blackberries as a valuable approach for its objective characterisation. Thus, only a single paper by D'Agostino et al. [17] has been previously reported on optimisation of a SPME followed by GC–MS method for its application to the characterisation of the volatile composition of *R. ulmifolius* Schott blackberries collected in 2012 in different locations of Calabria and, for comparison, in different regions all over Spain.

Table 3 lists the 71 volatiles of different functionality determined in the seven wild/cultivated Calabrian blackberries analysed, together with their retention data (I^T) and percent concentrations. As an example of the different chromatographic profiles obtained for each of the samples under study, Fig. 1 shows the total ion current (TIC) chromatograms of samples GRA (wild) and BOV (cultivated).

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Fig. 1 Volatile profiles obtained by SPME GC–MS for Calabrian blackberries GRA (wild) and BOV (cultivated). For peak identification, see Table 3

Table 3 Volatile compounds (mean and standard deviation in brackets, n = 3) determined in Calabrian *R. ulmifolius* Schott blackberries

Peak No	Compound	I^T	Relative data (%)						
			COS	FIL	ROS	CIC	GRA	NIC	BOV
1	Ethyl acetate	–	5.6^{c} (1.5)*	4.9^{d} (0.5)	4.6^{d} (7.6)	6.7^{b} (6.6)	6.6^{b} (1.9)	11.9^{a} (2.8)	5.8^{c} (0.2)
2	Methylbutanal (sum of isomers)	–	9.0^{b} (1.3)	7.1^{c} (1.3)	16.6^{a} (1.0)	6.5^{cd} (4.1)	7.2^{c} (9.9)	17.4^{a} (1.2)	5.7^{d} (9.0)
3	Ethanol	–	31.0^{a} (5.2)	25.2^{bc} (9.3)	11.8^{e} (12.2)	21.7^{cd} (6.9)	26.3^{b} (8.3)	20.5^{d} (3.4)	32.4^{a} (0.0)
4	2,3-Butanedione	–	6.4^{ab} (0.7)	6.2^{b} (0.2)	3.6^{e} (5.6)	6.7^{a} (1.8)	4.1^{d} (2.6)	3.5^{e} (3.7)	5.4^{c} (3.2)
5	Hexanal	1111	1.8^{c} (3.4)	1.2^{e} (8.3)	3.0^{a} (2.3)	1.5^{d} (4.3)	1.5^{de} (2.5)	2.6^{b} (5.9)	1.3^{de} (10.8)
6	2-Methyl-2-butenal	1118	0.1^{c} (7.8)	0.1^{d} (2.7)	0.1^{b} (1.1)	0.2^{a} (5.6)	0.1^{e} (4.5)	0.1^{b} (6.6)	0.1^{f} (17.8)
7	3-Penten-2-one	1141	0.8^{d} (2.6)	1.0^{cd} (22.0)	3.0^{a} (0.6)	2.1^{b} (14.4)	1.2^{c} (9.5)	1.2^{c} (2.6)	1.3^{c} (0.4)
8	1-Butanol	1158	1.3^{d} (0.9)	4.8^{a} (2.2)	1.2^{d} (2.1)	1.3^{d} (2.2)	2.6^{c} (1.1)	0.9^{d} (28.6)	3.4^{b} (18.4)
9	2-Heptanone	1188	2.5^{a} (1.3)	1.5^{b} (11.6)	0.9^{c} (2.7)	1.6^{b} (9.6)	0.7^{cd} (1.5)	0.6^{d} (17.6)	0.1^{e} (20.0)

10	Methyl hexanoate	1194	0.2^{d} (3.1)	0.9^{b} (11.4)	0.2^{d} (2.7)	1.5^{a} (6.9)	0.2^{d} (12.1)	0.5^{c} (18.4)	0.2^{d} (16.4)
11	Limonene	1197	tr^{b}**	tr^{b}	0.1^{b} (0.9)	0.1^{b} (33.3)	tr^{b}	tr^{b}	1.2^{a} (31.2)
12	1,8-Cineole	1203	tr^{b}	tr^{b}	0.1^{b} (7.2)	tr^{b}	0.1^{b} (0.5)	0^{b}	0.7^{a} (36.7)
13	3-Methyl-1-butanol	1213	0.6^{b} (3.3)	0.9^{a} (1.7)	0.3^{c} (7.8)	0.6^{b} (4.4)	0.6^{b} (4.2)	0.9^{a} (3.6)	0.2^{c} (52.6)
14	*trans*-2-Hexenal	1219	7.1^{c} (4.2)	3.0^{e} (0.7)	21.3^{a} (3.6)	6.3^{cd} (9.9)	5.3^{d} (18.4)	13.3^{b} (0.8)	2.7^{e} (26.0)
15	Ethyl hexanoate	1238	0.2^{b} (5.8)	0.3^{a} (12.5)	tr^{d}	0.3^{a} (13.5)	0.1^{bc} (4.1)	0.1^{c} (22.6)	tr^{d}
16	1-Pentanol	1255	0.2^{c} (2.7)	0.5^{a} (0.3)	0.4^{b} (13.6)	0.2^{c} (39.7)	0.4^{b} (3.2)	0.4^{b} (13.6)	0.6^{a} (9.3)
17	*p*-Cymene	1268	tr^{b}	tr^{b}	0.1^{b} (9.7)	0.1^{b} (13.6)	tr^{b}	0.1^{b} (19.6)	0.7^{a} (19.6)
18	3-Hydroxy-2-butanone	1289	1.6^{b} (11.1)	0.7^{d} (1.4)	0.8^{cd} (3.2)	2.0^{a} (9.7)	1.0^{c} (2.0)	1.3^{b} (1.8)	1.0^{c} (7.9)
19	2-Methylbutyl 3-methylbutanoate	1298	0.2^{a} (1.0)	0.1^{c} (15.2)	0.1^{d} (3.1)	0.1^{c} (4.0)	0.1^{b} (1.0)	0.1^{d} (15.3)	tr^{e}
20	4-Methyl-1-pentanol	1318	0.2^{c} (0.5)	0.2^{c} (23.8)	0.9^{a} (15.1)	–^{d}	0.4^{b} (3.1)	0.2^{c} (2.3)	0.2^{c} (12.6)
21	2-Heptanol	1325	1.5^{cd} (4.7)	1.8^{c} (6.2)	4.2^{a} (5.1)	1.5^{d} (9.7)	2.3^{b} (1.3)	1.3^{de} (4.2)	1.0^{e} (8.6)
22	6-Methyl-5-hepten-2-one	1339	0.1^{cde} (16.5)	0.1^{e} (8.0)	0.2^{b} (1.4)	0.1^{de} (26.5)	0.1^{bcd} (19.3)	0.2^{bc} (2.6)	0.3^{a} (8.7)
23	1-Hexanol	1357	7.8^{c} (0.8)	16.3^{a} (6.3)	1.7^{d} (2.5)	8.1^{c} (9.6)	9.6^{b} (0.8)	2.6^{d} (7.1)	2.7^{d} (5.0)
24	*trans*-3-Hexen-1-ol	1368	0.3^{a} (0.3)	0.1^{c} (20.6)	0.2^{b} (8.4)	0.1^{c} (6.9)	0.3^{a} (0.9)	0.3^{a} (14.1)	0.1^{c} (21.4)
25	Methyl octanoate	1391	tr^{c}	0.1^{c} (18.0)	0.2^{b} (7.0)	0.2^{b} (17.4)	0.1^{c} (4.1)	0.3^{a} (20.6)	0.1^{c} (16.7)
26	Nonanal	1393	2.0^{b} (0.5)	1.4^{bc} (8.9)	2.0^{b} (9.8)	2.0^{b} (24.1)	2.7^{a} (5.7)	1.2^{c} (3.6)	2.2^{a} (20.9)
27	2-Hexen-1-ol	1411	0.3^{cd} (2.0)	0.3^{c} (23.9)	0.7^{b} (0.5)	0.2^{d} (4.9)	0.7^{b} (9.7)	0.9^{a} (2.8)	0.2^{d} (6.8)
28	Hexyl butanoate	1417	0.1^{d} (0.7)	0.2^{c} (11.4)	0.1^{d} (12.6)	0.1^{d} (4.5)	0.6^{a} (1.9)	tr^{e}	0.2^{b} (6.6)
29	Ethyl octanoate	1437	0.1^{d} (2.0)	0.1^{d} (17.0)	0.2^{c} (5.7)	0.2^{b} (6.4)	0.2^{bc} (3.1)	0.3^{a} (14.6)	0.1^{d} (13.0)
30	*α*-Cubebene	1449	0.1^{c} (0.9)	0.1^{c} (19.5)	tr^{d}	0.2^{a} (6.7)	0.2^{b} (8.3)	0.2^{c} (15.3)	0.1^{a} (15.0)
31	Non-identified(43 (100), 45 (28), 58 (22), 84 (18), 69 (13))***	1456	0.4^{ef} (10.2)	0.7^{d} (4.5)	2.8^{a} (3.7)	1.6^{b} (10.6)	1.2^{c} (1.5)	0.5^{de} (12.2)	0.3^{f} (1.4)
32	1-Heptanol	1460	0.4^{b} (0.0)	0.6^{a} (9.6)	0.2^{cd} (1.2)	0.5^{a} (4.5)	0.6^{a} (3.7)	0.2^{c} (3.6)	0.2^{d} (0.8)
33	2-Furancarboxaldehyde	1467	0.6^{e} (2.3)	0.1^{f} (1.7)	3.5^{b} (6.0)	1.3^{d} (8.8)	2.0^{c} (10.4)	0.3^{ef} (4.8)	14.8^{a} (0.0)
34	*α*-Ylangene	1470	tr^{c}	tr^{c}	–^{d}	0.1^{b} (7.4)	0.1^{b} (2.5)	0.1^{a} (17.9)	tr^{c}
35	5,5-Dimethyl-2-cyclopenten-1-one	1474	0.1^{d} (4.3)	0.1^{e} (10.7)	0.6^{a} (0.6)	0.2^{b} (2.9)	0.2^{c} (6.1)	tr^{e}	0.1^{d} (14.7)
36	*α*-Copaene	1478	0.3^{d} (7.3)	0.2^{d} (14.4)	0.2^{d} (11.4)	0.6^{ab} (4.9)	0.5^{bc} (0.1)	0.7^{a} (17.0)	0.4^{c} (20.8)
37	Methyl 3-hydroxybutanoate	1484	–^{c}	tr^{b}	–^{c}	–^{c}	–^{c}	–^{c}	0.2 ^{a} (4.6)

38	2,4-Heptadienal	1491	0.1^{e} (11.4)	0.1^{e} (9.9)	0.2^{a} (4.9)	0.1^{c} (4.0)	0.1^{c} (7.2)	0.1^{d} (5.4)	0.1^{b} (2.7)
39	Decanal	1497	0.2^{c} (2.8)	0.1^{c} (15.4)	0.4^{a} (10.9)	0.3^{b} (27.4)	0.2^{bc} (4.4)	0.4^{a} (0.4)	–^{d}
40	Camphor	1498	tr^{b}	tr^{b}	tr^{b}	tr^{b}	tr^{b}	tr^{b}	1.1^{a} (5.4)
41	3-Ethyl-4-methylpentanol	1512	0.1^{cd} (11.4)	0.1^{d} (2.7)	0.1^{b} (8.6)	0.1^{c} (10.1)	0.1^{b} (6.4)	0.3^{a} (7.9)	0.1^{d} (6.1)
42	Benzaldehyde	1517	0.3^{ab} (8.6)	0.2^{d} (1.3)	0.3^{a} (4.9)	0.2^{cd} (11.6)	0.3^{abc} (14.2)	0.2^{d} (3.6)	0.3^{bcd} (10.6)
43	Ethyl 3-hydroxybutanoate	1520	tr^{d}	0.1^{b} (7.9)	tr^{d}	tr^{e}	tr^{c}	–^{e}	0.7^{a} (0.3)
44	Epizonarene	1536	0.1^{b} (9.4)	0.1^{b} (11.8)	tr^{c}	0.3^{a} (4.3)	0.3^{a} (1.8)	0.3^{a} (14.2)	0.1^{b} (13.9)
45	Linalool	1554	0.3^{ab} (23.7)	0.2^{bc} (5.9)	0.4^{a} (17.3)	0.2^{c} (28.1)	0.2^{bc} (21.1)	0.3^{b} (6.8)	0.3^{b} (2.3)
46	1-Octanol	1562	6.7^{c} (8.0)	8.8^{b} (4.6)	2.1^{e} (9.4)	11.6^{a} (1.9)	9.2^{b} (4.2)	3.1^{d} (4.2)	2.7^{de} (0.6)
47	5-Methylfurfural	1572	tr^{c}	tr^{c}	0.1^{b} (9.9)	0.1^{b} (31.7)	0.1^{b} (6.7)	tr^{c}	0.4^{a} (0.3)
48	Methyl decanoate	1596	0.1^{b} (13.8)	tr^{c}	0.1^{a} (10.1)	0.1^{b} (9.4)	0.1^{b} (6.8)	0.1 ^{a} (5.5)	0.1^{a} (5.1)
49	Hexyl hexanoate	1611	0.2^{d} (12.4)	0.2^{c} (3.7)	0.1^{e} (23.9)	0.2^{c} (9.3)	0.5^{a} (0.7)	tr^{f}	0.3^{b} (1.6)
50	Dihydro-2(3H)-furanone	1617	0.5^{e} (2.9)	0.5^{ef} (2.9)	0.8^{d} (3.4)	1.1^{b} (10.9)	0.9^{c} (2.6)	0.3^{f} (14.6)	2.0^{a} (4.4)
51	Phenylacetaldehyde	1638	0.5^{bc} (11.6)	0.2^{e} (3.8)	0.9^{a} (8.7)	0.4^{cd} (21.7)	0.3^{de} (12.8)	0.6^{b} (9.9)	0.4^{cd} (4.3)
52	Ethyl decanoate	1640	0.3^{a} (14.7)	0.1^{d} (5.2)	0.1^{c} (18.4)	0.2^{b} (11.2)	0.2^{b} (0.6)	0.2^{b} (1.9)	0.1^{cd} (0.2)
53	1-Nonanol	1664	0.5^{bc} (9.1)	0.7^{a} (2.7)	0.6^{b} (11.9)	0.5^{bc} (12.7)	0.9^{a} (2.6)	0.3^{c} (3.7)	0.4^{bc} (38.0)
54	1-Methoxy-4-(2-propenyl)-benzene	1665	tr^{b}	0.6^{a} (18.5)	tr^{b}	tr^{b}	tr^{b}	tr^{b}	tr^{b}
55	2-Furanmethanol	1667	0.1^{d} (6.3)	tr^{d}	0.2^{b} (16.6)	0.1^{c} (14.7)	0.2^{b} (8.1)	0.1^{d} (4.6)	0.9^{a} (1.5)
56	*α*-Terpineol	1693	0.1^{cd} (5.6)	0.1^{cd} (3.6)	0.1^{c} (3.7)	tr^{d}	0.1^{b} (9.4)	0.1^{cd} (0.7)	0.2^{a} (10.0)
57	Methyl butanoic acid	1710	4.2^{c} (1.4)	4.4^{c} (4.5)	4.1^{c} (1.8)	4.8^{b} (4.0)	2.8^{d} (2.2)	6.7^{a} (2.0)	1.0^{e} (0.6)
58	Cadinene	1746	tr^{cd}	tr^{de}	tr^{e}	0.1^{a} (16.7)	tr^{c}	tr^{b}	0.1^{a} (1.1)
59	1-Decanol	1767	0.3^{de} (11.4)	0.5^{bc} (2.1)	0.4^{cd} (19.6)	0.8^{a} (13.8)	0.6^{b} (3.0)	0.3^{e} (0.9)	0.4^{cd} (3.9)
60	Myrtenol	1788	tr^{c}	0.1^{bc} (32.1)	0.1^{bc} (2.2)	tr^{c}	tr^{c}	0.1^{b} (54.5)	0.2^{a} (12.5)
61	Methyl dodecanoate	1804	0.2^{b} (24.9)	0.1^{c} (3.6)	0.1^{bc} (15.3)	0.1^{b} (17.0)	0.1^{bc} (2.9)	0.1^{c} (10.0)	0.3^{a} (12.1)
62	1-Methoxy-4-(1-propenyl)-benzene	1822	tr^{c}	0.3^{a} (7.3)	tr^{c}	tr^{c}	0.1^{b}	–^{c}	tr^{c}
63	Ethyl dodecanoate	1845	0.2^{a} (17.9)	0.1^{c} (0.1)	tr^{cd}	0.1^{bc} (28.7)	0.1^{b} (5.6)	tr^{cd}	–^{d}
64	*p*-Cymen-8-ol	1849	–^{b}	–^{b}	–^{b}	–^{b}	–^{b}	–^{b}	0.6^{a} (18.8)
65	Benzyl alcohol	1874	1.6^{ab}	1.0^{d}	1.8^{a}	0.9^{d}	1.3^{bc}	1.0 cd	0.5^{e}

			(14.9)	(2.0)	(12.8)	(11.0)	(3.1)	(6.5)	(0.6)
66	2-Phenylethanol	1906	0.4^{cd} (17.9)	0.5^{c} (2.5)	0.8^{b} (15.7)	0.9^{ab} (12.0)	1.0^{a} (2.7)	0.8^{ab} (7.4)	0.3^{d} (4.4)
67	2-Methyl-3-phenyl-1-propanol	1988	0.1^{a} (17.3)	0.1^{b} (2.8)	tr^{cd}	tr^{cd}	0.1^{c} (3.6)	tr^{cd}	tr^{cd}
68	4-Ethyl-2-methoxyphenol	2026	$–^{c}$	0.1^{b} (2.9)	$–^{c}$	tr^{c}	0.1^{a} (13.9)	tr^{c}	$–^{c}$
69	Non-identified(112 (100), 55 (98), 142 (85), 84 (85), 56 (46), 117 (35))***	2038	0.2^{c} (0.6)	0.1^{d} (2.2)	0.4^{a} (20.9)	0.2^{c} (16.3)	0.3^{bc} (5.5)	0.1^{d} (8.1)	0.3^{ab} (5.4)
70	Benzenepropanol	2040	0.2^{a} (19.9)	0.1^{b} (7.0)	0.1^{c} (19.3)	0.1^{b} (14.3)	0.1^{b} (4.8)	tr^{c}	tr^{c}
71	5-Hydroxymethylfurfural	2498	$–^{b}$	$–^{b}$	0.1^{b} (92.8)	0.1^{b} (120.5)	tr^{b}	$–^{b}$	0.2^{a} (15.7)

*Entries for each compound followed by the same letter showed no statistically significant differences for their mean value at the 95% confidence level;
**tr = trace (< 0.01%);
***Mass spectrum: *m/z* and abundance (%) in brackets

Although a wide variability in the qualitative and quantitative composition was observed regarding the collection place of wild blackberries, compounds with the highest concentrations were common to both wild and cultivated blackberries: ethanol (11.8–32.4%), methylbutanal (sum of isomers) (5.7–17.4%), ethyl acetate (4.6–11.9%), 2,3-butanedione (3.5–6.7%), *trans*-2-hexenal (2.7–21.3%), 1-hexanol (1.7–16.3%), 1-octanol (2.1–11.6%), and methyl butanoic acid (1.0–6.7%). Except for some volatiles (e.g. 1-butanol in FIL and BOV samples; 2-heptanol in ROS, etc.), the remaining compounds were present in concentrations lower than 3%. Esters, aliphatic alcohols and terpenoids were the predominant classes, followed by aldehydes, aromatic alcohols, ketones and furan derivatives. Regarding the harvest location, FIL sample showed the richest volatile composition followed by COS and NIC samples.

In agreement with data previously reported by D'Agostino et al. [17] for *R. ulmifolius* Schott blackberries collected both in Calabria (Italy) and in different provinces of Spain in 2012, Calabrian blackberries here studied and collected in 2011 were characterised by the presence of a number of aliphatic esters including methyl and ethyl esters of C_6-C_{12} acids, hexyl butanoate and hexanoate, etc. (Table 3). The harvest year was also shown to exert an effect on volatile percent concentrations. Ethyl acetate, one of the most abundant compounds in this study, was only present in concentrations up to 3.93% in some of Calabrian blackberries collected in 2012 [17]. However, relative concentrations of other volatiles such as hexyl butanoate (peak 28) and 3-hydroxy esters (peaks 37 and 43) were shown to be higher in **samples** collected in Granatara and Bovalino, respectively, irrespective of the harvest year. Aromatic esters, reported as major components of other *Rubus* species [5, 15] were not detected in Calabrian blackberries here analysed.

Aliphatic alcohols have been described as one of the predominant classes in different *Rubus* blackberries [1, 3, 43, 44]. In agreement with data for 2012 blackberries reported by D'Agostino et al. [17], and irrespective of the harvest location and sample type (wild/cultivated), high levels of ethanol were determined in all Calabrian *R. ulmifolius* Schott blackberries here studied. The inspection of the samples prior to analysis regarding the quality and homogeneity of their ripening stage, together with the appropriate preservation process followed, ruled out the spoilage or bacterial growth as the origin of this compound. Moreover, the presence of ethanol as a natural component of the aroma of a number of food matrices, including red fruits, has been previously described [17, 45].

As for the harvest year, 4-methyl-1-pentanol (peak 20) was consistently higher in ROS samples, as compared to other Calabrian locations. A wide variability was also determined in the 2-heptanol (peak 21) content of Calabrian blackberries, with ROS sample showing the highest concentration (4.2%), and NIC and BOV samples, the lowest (~ 1.0%). In a study by Qian and Wang [44] on cultivated blackberries, concentration of this alcohol was shown to change differently with the cultivar considered; 'Thornless Evergreen' showed a significantly higher concentration over 'Marion' blackberries. High levels of 2-heptanol and of 1-terpinen-4-ol have also been reported to be distinctive of *R. glaucus* Benth. samples analysed by SPME [5, 15].

As previously stated in introduction, blackberry aroma depends on a number of factors (e.g. harvesting

location and time, cultivar, etc.) and it is the result of a wide variety of volatiles present in different concentrations, each of them providing a diversity of aromatic notes (esters: floral, fruity and sweet; alcohols: floral, fruity and green, etc.) [44]. Although the low number of Calabrian samples available for this study make not possible to draw any definitive conclusion, the absence or the presence of a few volatiles seemed to be characteristic of the samples here analysed. As for wild samples, 1-hexanol, 1-methoxy-4-(2-propenyl)-benzene and 1-methoxy-4-(1-propenyl)-benzene were typical of FIL samples. In addition to 4-methyl-1-pentanol, methylbutanal, 3-penten-2-one, *trans*-2-hexenal, and 2-heptanol were detected at higher relative concentrations in ROS sample. Concentrations of methylbutanal as high as 17.4% and similar to those of ROS blackberries were also detected in NIC blackberries. Methyl 3-hydroxybutanoate (0.2%), camphor (1.1%), and *p*-cymen-8-ol (0.6%) were only detected in BOV sample, whereas decanal was only present in Calabrian wild blackberries (0.1–0.4%). Other minor compounds present in a significantly different concentration in BOV sample were 2-heptanone (0.1%), limonene (1.2%), 1,8-cineole (0.7%), *p*-cymene (0.7%), and ethyl 3-hydroxybutanoate (0.7%). As shown in Table 3, the level of furan derivatives was also significantly higher in the cultivated sample over the wild blackberries. The different pre-harvesting and post-harvesting conditions might justify these differences.

Total anthocyanins, total phenolics and antioxidant capacity

Table 4 lists the results obtained for total anthocyanins, total phenolics and antioxidant capacity, as determined by both DPPH and ABTS assays, of the wild and cultivated Calabrian blackberries under study.

Table 4 Antioxidant capacity (data are reported as mean ± standard deviation, $n = 3$) of Calabrian *R. ulmifolius* Schott blackberries

Samples	TAC (mg Cyd-3-Glu equivalents g^{-1} DW)	TPC (mg GAE g^{-1} DW)	DPPH SE_{50} (mg DW)	ABTS (μmol trolox g^{-1} DW)
COS	$12.04 \pm 0.08^{a*}$	41.46 ± 0.11^{a}	1.65 ± 0.04^{a}	24.77 ± 0.05^{a}
FIL	6.08 ± 0.36^{d}	26.33 ± 0.23^{e}	3.36 ± 0.04^{c}	10.03 ± 0.03^{d}
ROS	8.68 ± 0.02^{b}	28.00 ± 0.14^{d}	2.19 ± 0.01^{b}	11.66 ± 0.02^{c}
CIC	12.47 ± 0.06^{a}	34.24 ± 0.01^{b}	1.67 ± 0.01^{a}	23.64 ± 0.02^{a}
GRA	10.76 ± 0.03^{bc}	44.61 ± 0.39^{a}	2.88 ± 0.03^{b}	16.15 ± 0.01^{b}
NIC	7.33 ± 0.06^{c}	31.74 ± 0.50^{c}	2.21 ± 0.01^{b}	10.21 ± 0.02^{d}
BOV	4.64 ± 0.01^{e}	17.90 ± 0.07^{f}	7.84 ± 0.02^{d}	4.99 ± 0.01^{e}

*Entries for TAC/TPC/DPPH/ABTS followed by the same letter showed no statistically significant differences for their mean value at the 95% confidence level

TAC of the cultivated sample (BOV) was significantly lower (4.64 mg Cyd-3-Glu equivalents g^{-1} DW) than those of wild blackberries (6.08–12.47 mg Cyd-3-Glu equivalents g^{-1} DW or 1.56–2.76 mg Cyd-3-Glu g^{-1} FW). As compared to the literature, TAC of the cultivated BOV sample (0.71 mg Cyd-3-Glu equivalents g^{-1} FW) was found to be within the range (0.67–2.11 mg Cyd-3-Glu equivalents g^{-1} FW) previously reported for different cultivars of *Rubus* sp. berries [13, 41, 42, 46]. Regarding the harvest location of wild samples, COS and CIC blackberries showed the highest TAC level (> 12 mg Cyd-3-Glu equivalents g^{-1} DW).

Similarly, total phenolic content of Calabrian wild blackberries (26.33–44.61 mg GAE g^{-1} DW or 5.33–9.06 mg GAE g^{-1} FW) was significantly higher than that of the cultivated sample BOV (17.9 mg GAE g^{-1} DW) and other cultivated blackberries collected in Italy (2.37–3.17 mg GAE g^{-1} FW) [46] and all over the world: 12.14–20.61 mg GAE g^{-1} DW [13, 36, 41] or 1–1.3 mg g^{-1} FW [39]. The high TPC of wild samples COS and GRA was similar to that reported for wild *R. adenotrichus* (42.5 mg GAE g^{-1} DW) from Costa Rica but lower than that of *R. glaucus* Benth. from Ecuador (63 mg GAE g^{-1} DW) [19]. In agreement with the results above shown for Calabrian samples, Koca and Karadeniz [21] reported a higher content of both total anthocyanins (1.30–1.97 mg Cyd-3-Glu equivalents $^{-1}$ FW) and total phenolics (2.64–3.79 mg GAE g^{-1} FW) in wild blackberries collected in Turkey, as compared to cultivated samples (TAC: 0.95–1.58 mg Cyd-3-Glu equivalents g^{-1} FW; TPC: 1.73–3.05 mg GAE g^{-1} FW).

As regard as the radical scavenging activity of Calabrian blackberries, all samples exhibited a significant

antioxidant capacity (DPPH assay), expressed as SE_{50} values (wild samples: 0.36–0.68 mg FW; cultivated sample: 1.20 mg FW). As compared with data reported in the literature for pistachio (*Pistacia vera* L., variety Bronte) seeds and skins (SE_{50} = 14.99 and 0.019 mg FW, respectively) [32], and for natural and blanched almond skins (SE_{50} = 0.24 and 5.25 mg DW, respectively) [47], blackberries are shown as a rich dietary source of antioxidants. Data reported in different studies on antioxidant activity of red fruits cultivated in Potenza (Southern Italy) [46], and in Thessaloniki (Northern Greece) [13], would also confirm these results.

As TAC and TPC, wild blackberries showed a higher bioactivity measured by the DPPH assay (SE_{50} = 1.65–3.36 mg DW) than the cultivated sample (SE_{50} = 7.84 mg DW), with samples collected in Cosoleto and Cicerna showing the highest bioactivity. Similar conclusions were also drawn from ABTS results, despite its different radical scavenging mechanism (single electron transfer (SET) *vs* SET and hydrogen atom transfer of DPPH) [48]. The highest antioxidant capacity was found for wild samples (ranging between 10.03 and 24.77 μmol trolox g^{-1} DW for FIL and COS, respectively), whereas the cultivated blackberries accounted for 4.99 μmol trolox g^{-1} DW (Table 4).

In the literature, the antioxidant activity of blackberries has been reported to be strongly correlated with total phenolics [13, 25] or with total anthocyanins [13, 15, 21, 41]. In our study, when TAC and TPC were individually considered, a high ($r = -0.7$) inverse correlation of both parameters with DPPH results was found. A non-significant increase in the correlation ($r = -0.76$, $P < 0.2$) was observed when considering both parameters together. TAC and TPC values were significantly correlated ($r = 0.84$) at the 95% confidence level, and the positive correlation of each of them with ABTS results was $r = 0.96$ and $r = 0.77$, respectively. These results confirm the complementarity of the methods here used for the evaluation of the bioactivity of Calabrian blackberries.

Finally, although previous studies have reported the influence of sample treatment (e.g. grinding) on total anthocyanin, total phenolics and AC results [49, 50], the fact that samples under study have been subjected to identical procedure make the results of Table 4 useful for the comparison purposes here intended.

Conclusion

The results on physicochemical data, antioxidant activity and volatile composition described in this paper are a contribution to the comprehensive characterisation of *Rubus ulmifolius* Schott blackberries from Calabria, and can also be very valuable for the establishment of standards of quality ~~in order~~ to revalorise and promote the commercialisation of this product. Remarkably, the higher TAC, TPC and AC of Calabrian wild blackberries with respect to the cultivated fruits could be a claim for consumers demanding the bioactive properties of blackberries. The higher dry matter content of wild fruits reduces their perishability and the higher pH and lower total acidity increase the acceptability for consumers.

Although no single volatile may be considered a quality marker, it is generally accepted that cultivated foods (including blackberries) resembling the sensorial properties of wild samples are usually preferred by consumers. Therefore, the comparison in this study of the multicomponent volatile profile of both wild and cultivated blackberries, and its consistency with harvesting year, represent a valuable approach for the objective characterization of Calabrian blackberries here intended. Moreover, the correlation of these chemical data with results from sensory analysis would be very useful for selecting the optimal location for collection of wild blackberries as well as for breeding programs addressed to the development of blackberry cultivars with improved aroma and bioactivity.

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ArticleNote

Acknowledgements

This work was conducted in the ambit of the 3-year PhD (XXVI cycle) of Maria Francesca D'Agostino with the title of Doctor Europaeus in: Biology Applied to Agro-food and Forestry Systems. Title of the PhD thesis: 'Antioxidant activity and volatile composition of *Rubus ulmifolius* Schott blackberries from Calabria (Italy)'. FundingThis work is part of the I + D + I project PID2019-106405 GB-I00 financed by MCIN/AEI/ https://doi.org/10.13039/501100011033. Authors also thank the Comunidad of Madrid and European funding from FSE and FEDER programs (project S2018/BAA-4393, AVANSECAL-II-CM) and Fundación Ramón Areces (project CIVP17A2843) for financial support. M.F.D. thanks the Università degli Studi *Mediterranea* di Reggio Calabria for her fellowship grant.

Declarations

Conflict of interest

Authors declare no conflict of interest.

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