# Dear Author

Here are the proofs of your article.

- You can submit your corrections **online** or by **fax**.

- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.

- For **fax** submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.

- Together with the proof please return the cover sheet (including the *Copyright Transfer Statement)* and the *Offprint Order Form.* They can either be scanned and sent electronically or sent by fax.

- Remember to note the journal title, article number, and your name when sending your response via e-mail, fax or regular mail.

- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.

- **Check** the questions that may have arisen during copy editing and insert your answers/corrections.

- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.

- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.

- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style.
  Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.

- If we do not receive your corrections **within 48 hours**, we will send you a reminder.

**Please note**

Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL:

```
http://dx.doi.org/10.1007/s00216-016-0067-y
```

If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information, go to:
http://www.link.springer.com.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us, if you would like to have these documents returned.

The **printed version** will follow in a forthcoming issue.

---

# Offprint Order Form

- To determine if your journal provides free offprints, please check the journal's instructions to authors.
- **If you do not return this order form,** we assume that **you do not wish to order offprints.**
- If you order offprints **after** the issue has gone to press, costs are much higher. Therefore, we can supply offprints only in quantities of 300 or more after this time.

- For orders involving more than 500 copies, please ask the production editor for a quotation.

---

**Please enter my order for:**

| Pages | 1-4 | 1-4 | 5-8 | 5-8 | 9-12 | 9-12 | 13-16 | 13-16 | 17-20 | 17-20 | 21-24 | 21-24 | 25-28 | 25-28 | 29-32 | 29-32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Copies | EUR | USD | EUR | USD | EUR | USD | EUR | USD | EUR | USD | EUR | USD | EUR | USD | EUR | USD |
| ❏ 50 | 250.00 | 275.00 | 300.00 | 330.00 | 370.00 | 405.00 | 430.00 | 475.00 | 500.00 | 550.00 | 525.00 | 575.00 | 575.00 | 630.00 | 610.00 | 670.00 |
| ❏ 100 | 300.00 | 330.00 | 365.00 | 405.00 | 465.00 | 510.00 | 525.00 | 580.00 | 625.00 | 685.00 | 655.00 | 720.00 | 715.00 | 785.00 | 765.00 | 840.00 |
| ❏ 200 | 400.00 | 440.00 | 525.00 | 575.00 | 645.00 | 710.00 | 740.00 | 815.00 | 860.00 | 945.00 | 925.00 | 1,015.00 | 1,005.00 | 1,105.00 | 1,105.00 | 1,190.00 |
| ❏ 300 | 500.00 | 550.00 | 680.00 | 750.00 | 825.00 | 910.00 | 955.00 | 1,050.00 | 1,095.00 | 1,205.00 | 1,190.00 | 1,310.00 | 1,295.00 | 1,425.00 | 1,425.00 | 1,530.00 |
| ❏ 400 | 610.00 | 670.00 | 855.00 | 940.00 | 1,025.00 | 1,130.00 | 1,195.00 | 1,315.00 | 1,360.00 | 1,495.00 | 1,485.00 | 1,635.00 | 1,615.00 | 1,775.00 | 1,775.00 | 1,915.00 |
| ❏ 500 | 720.00 | 790.00 | 1,025.00 | 1,130.00 | 1,225.00 | 1,350.00 | 1,430.00 | 1,575.00 | 1,625.00 | 1,780.00 | 1,780.00 | 1,960.00 | 1,930.00 | 2,125.00 | 2,090.00 | 2,300.00 |

**Orders will only be processed if a credit card number has been provided. For German authors, payment by direct debit is also possible.**

I wish to be charged in     ❏ Euro    ❏ USD

Prices include surface mail postage and handling.
Customers in EU countries who are not registered for VAT should add VAT at the rate applicable in their country.

VAT registration number (EU countries only):

_____

**Please charge my credit card**
- ❏ **Eurocard/Access/Mastercard**
- ❏ **American Express**
- ❏ **Visa/Barclaycard/Americard**

**For authors resident in Germany: payment by direct debit:**
**I authorize Springer to debit the amount owed from my bank account at the due time.**

**Number (incl. check digits):**

_ _ _ _  _ _ _ _  _ _ _ _  _ _ _ _  _ _ _

**Valid until:** _ _ / _ _

**Date / Signature:** _____

**Account no.:** _____

**Bank code:** _____

**Bank:** _____

**Date / Signature:** _____

---

**Send receipt to:**

❏ Massimo Bubba
Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019, Sesto Fiorentino, Florence, Italy

**Ship offprints to:**

❏ Massimo Bubba
Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019, Sesto Fiorentino, Florence, Italy

❏ _____
_____
_____
_____
_____

❏ _____
_____
_____
_____
_____

# Metadata of the article that will be visualized in OnlineFirst

| 1 | Article Title | | **Liquid chromatographic/electrospray ionization quadrupole/time of flight tandem mass spectrometric study of polyphenolic composition of different *Vaccinium* berry species and their comparative evaluation** |
|---|---|---|---|
| 2 | Article Sub-Title | | |
| 3 | Article Copyright - Year | | **Springer-Verlag Berlin Heidelberg 2016 (This will be the copyright line in the final PDF)** |
| 4 | Journal Name | | Analytical and Bioanalytical Chemistry |
| 5 | | Family Name | **Bubba** |
| 6 | | Particle | **Del** |
| 7 | | Given Name | **Massimo** |
| 8 | Corresponding Author | Suffix | |
| 9 | | Organization | University of Florence |
| 10 | | Division | Department of Chemistry |
| 11 | | Address | Via della Lastruccia 3, Sesto Fiorentino 50019, Florence |
| 12 | | e-mail | delbubba@unifi.it |
| 13 | | Family Name | **Ancillotti** |
| 14 | | Particle | |
| 15 | | Given Name | **Claudia** |
| 16 | | Suffix | |
| 17 | Author | Organization | University of Florence |
| 18 | | Division | Department of Chemistry |
| 19 | | Address | Via della Lastruccia 3, Sesto Fiorentino 50019, Florence |
| 20 | | e-mail | |
| 21 | | Family Name | **Ciofi** |
| 22 | | Particle | |
| 23 | | Given Name | **Lorenzo** |
| 24 | Author | Suffix | |
| 25 | | Organization | University of Florence |
| 26 | | Division | Department of Chemistry |
| 27 | | Address | Via della Lastruccia 3, Sesto Fiorentino 50019, Florence |

| 28 | | e-mail | |
|----|--------|-------------|----|
| 29 | | Family Name | **Rossini** |
| 30 | | Particle | |
| 31 | | Given Name | **Daniele** |
| 32 | | Suffix | |
| 33 | Author | Organization | University of Florence |
| 34 | | Division | Department of Chemistry |
| 35 | | Address | Via della Lastruccia 3, Sesto Fiorentino 50019, Florence |
| 36 | | e-mail | |
| 37 | | Family Name | **Chiuminatto** |
| 38 | | Particle | |
| 39 | | Given Name | **Ugo** |
| 40 | | Suffix | |
| 41 | Author | Organization | Sciex Europe |
| 42 | | Division | |
| 43 | | Address | Landwehrstraße 54, Darmstadt 64293 |
| 44 | | e-mail | |
| 45 | | Family Name | **Stahl-Zeng** |
| 46 | | Particle | |
| 47 | | Given Name | **Jianru** |
| 48 | | Suffix | |
| 49 | Author | Organization | Sciex Europe |
| 50 | | Division | |
| 51 | | Address | Landwehrstraße 54, Darmstadt 64293 |
| 52 | | e-mail | |
| 53 | | Family Name | **Orlandini** |
| 54 | | Particle | |
| 55 | | Given Name | **Serena** |
| 56 | | Suffix | |
| 57 | Author | Organization | University of Florence |
| 58 | | Division | Department of Chemistry |
| 59 | | Address | Via della Lastruccia 3, Sesto Fiorentino 50019, Florence |
| 60 | | e-mail | |
| 61 | | Family Name | **Furlanetto** |
| 62 | Author | Particle | |
| 63 | | Given Name | **Sandra** |

| 64 | | Suffix | |
| 65 | | Organization | University of Florence |
| 66 | | Division | Department of Chemistry |
| 67 | | Address | Via della Lastruccia 3, Sesto Fiorentino 50019, Florence |
| 68 | | e-mail | |

| 72 | Abstract | Ultra-high-performance liquid chromatography coupled with high-resolution quadrupole-time of flight mass spectrometry with both negative and positive ionization was used for comprehensively investigating the phenolic and polyphenolic compounds in berries from three spontaneous or cultivated *Vaccinium* species (i.e., *Vaccinium myrtillus*, *Vaccinium uliginosum* subsp. *gaultherioides*, and *Vaccinium corymbosum*). More than 200 analytes, among phenolic and polyphenolic compounds belonging to the classes of anthocyanins, monomeric and oligomeric flavonols, flavanols, dihydrochalcones, phenolic acids, together with other polyphenolic compounds of mixed structural characteristics, were identified. Some of the polyphenols herein investigated, such as anthocyanidin glucuronides and malvidin-feruloyl-hexosides in *V. myrtillus*, or anthocyanindin aldopentosides and coumaroyl-hexosides in *V. uliginosum* subsp. *gaultherioides* and a large number of proanthocyanidins with high molecular weight in all species, were described for the first time. Principal component analysis applied on original LC-TOF data, acquired in survey scan mode, successfully discriminated the three *Vaccinium* species investigated, on the basis of their polyphenolic composition, underlying one more time the fundamental role of mass spectrometry for food characterization. |

## Electronic supplementary material

**ESM 1**
(PDF 1213 kb)

1
3
2

**RESEARCH PAPER**

# Liquid chromatographic/electrospray ionization quadrupole/time of flight tandem mass spectrometric study of polyphenolic composition of different *Vaccinium* berry species and their comparative evaluation

**Claudia Ancillotti**[1] · **Lorenzo Ciofi**[1] · **Daniele Rossini**[1] · **Ugo Chiuminatto**[2] · **Jianru Stahl-Zeng**[2] · **Serena Orlandini**[1] · **Sandra Furlanetto**[1] · **Massimo Del Bubba**[1]

**Abstract** Ultra-high-performance liquid chromatography coupled with high-resolution quadrupole-time of flight mass spectrometry with both negative and positive ionization was used for comprehensively investigating the phenolic and polyphenolic compounds in berries from three spontaneous or cultivated *Vaccinium* species (i.e., *Vaccinium myrtillus*, *Vaccinium uliginosum* subsp. *gaultherioides*, and *Vaccinium corymbosum*). More than 200 analytes, among phenolic and polyphenolic compounds belonging to the classes of anthocyanins, monomeric and oligomeric flavonols, flavanols, dihydrochalcones, phenolic acids, together with other polyphenolic compounds of mixed structural characteristics, were identified. Some of the polyphenols herein investigated, such as anthocyanidin glucuronides and malvidin-feruloyl-hexosides in *V. myrtillus*, or anthocyanindin aldopentosides and coumaroyl-hexosides in *V. uliginosum* subsp. *gaultherioides* and a large number of proanthocyanidins with high molecular weight in all species, were described for the first time. Principal component analysis applied on original LC-TOF data, acquired in survey scan mode, successfully discriminated the three *Vaccinium* species investigated, on the basis of their polyphenolic composition, underlying one more time the fundamental role of mass spectrometry for food characterization.

✉ Massimo Del Bubba
delbubba@unifi.it

1 Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy

2 Sciex Europe, Landwehrstraße 54, 64293 Darmstadt, Germany

## Introduction

The consumption of berries (e.g., blackberry, bilberry, blueberry, and cranberry) is considered an important contribution to healthy diets, owing to the various classes of phenolic compounds contained in large quantities in these fruits [1]. In fact, the class of phenolic compounds comprises a very high and increasing number of bioactive compounds [2], which are suggested to provide important health-protecting attributes such as anti-inflammatory, antihypertensive, antimicrobial, and anticancer properties [3].

Among the different berry species, *Vaccinium myrtillus* is the wild bilberry native to mountain areas of Northern and Central Europe, widely diffused also in Italian Alps and Apennines. In these zones, the increasing presence of a different spontaneous *Vaccinium* species, recently identified through genetic analyses as the *Vaccinium uliginosum* subsp. *gaultherioides* (locally named "false bilberry"), has been recently observed [4]. The cultivation and commercialization of *Vaccinium corymbosum* berries (i.e., the blueberry) is also widespread in the same area.

*V. myrtillus* is one of the richest fruit in polyphenols, with particular regard to anthocyanins [5] and is therefore considered a "functional food" [6]. Accordingly, *V. myrtillus* berries are largely consumed both as fresh fruits and processed products, such as juices and dietary supplements.

Many researches focusing on the determination of selected anthocyanins were carried out on bilberries from different European areas [7–11]. Interestingly, the composition of the

most abundant anthocyanins (i.e., glucosides, galactosides, and arabinosides of cyanidin, delphinidin, petunidin, peonidin, and malvidin) of *V. myrtillus* berries has been found different from the ones of blueberry [7], suggesting the potential use of polyphenolic profiles for the discrimination of transformed products from these fruit species. This aspect is very important since *V. myrtillus* is supposed to be a food with a higher nutraceutical value than *V. corymbosum* [6].

Furthermore, the feasibility of using the anthocyanin profile as a species fingerprint becomes noteworthy for discerning *V. myrtillus* from *V. uliginosum* L. subsp. *gaultherioides*. In fact, the phenotype of this latter berry is very similar to the *V. myrtillus* one, and the two fruits might be confused by the harvesters involved in the production chain of transformed bilberry. Italian *V. uliginosum* L. subsp. *gaultherioides* fruits were recently analyzed for the first time by our team, evidencing a lower content of total soluble polyphenols and total monomeric anthocyanins, as well as smaller antioxidant and antiradical activities, compared to *V. myrtillus* ones [4]. Hence, from this point of view, a lower nutraceutical value of "false bilberry," compared to bilberry, can be assumed. Concentrations of individual anthocyanins found in "false bilberry" were in most cases lower than those of bilberry, as well. Moreover, the relative abundance of the predominant anthocyanins of *V. uliginosum* L. subsp. *gaultherioides* berries was found very different from that of *V. myrtillus* fruits [4] and, interestingly, rather similar to the profile of *V. corymbosum*, being for instance both "false bilberry" and blueberry characterized by the predominance of malvidin derivatives [4, 7].

The analysis of further classes of polyphenols, such as flavonols, flavanols, and phenolic acids, which might be also important for discriminating one *Vaccinium* species from another, has been performed only occasionally in *V. myrtillus* [12, 13] and *V. corymbosum* berries [14]. Data concerning some phenolic compounds have been recently reported also for *V. uliginosum* L. subsp. *gaultherioides* berries [4].

Nevertheless, in the current literature, there is a lack of indepth studies dealing with the simultaneous investigation of the different polyphenolic classes in *V. myrtillus*, *V. corymbosum*, and *V. uliginosum* L. subsp. *gaultherioides* berries.

In order to carry out such a kind of studies, complex analytical approaches, involving nontarget metabolomic investigations, are required. These investigations are commonly performed using liquid chromatography (LC) coupled with mass spectrometry (MS) [15, 16], employing in some cases also ultraviolet detection [17, 18] and occasionally nuclear magnetic resonance, as well [19]. Actually, LC-MS is one of the most powerful analytical technique for polyphenol analysis. In fact, atmospheric pressure ionization sources provide a soft ionization of target analytes, which is particularly recommended for structure elucidation of polar, nonvolatile, and thermally labile compounds, such as flavonoids. Moreover, the use of tandem mass spectrometry (MS/MS) enables to

obtain important structurally related information through the fragmentation of parent molecules. In this context, the adoption of high-resolution mass spectrometry (e.g., time-of-flight-based instruments) allows for obtaining accurate mass readout, thus facilitating the assignment of an elemental formula to the parent molecule and/or to the fragments and its fragmentation characteristics [20].

Based on the above-reported considerations, this study aimed at comprehensively investigating the polyphenolic profiles of *V. myrtillus*, *V. corymbosum*, and *V. uliginosum* L. subsp. *gaultherioides* berries through a nontarget LC-MS/MS approach, using a quadrupole/time of flight mass spectrometry (Q/TOF).

## Material and methods

### Reagents and standards

Polyphenol standards were supplied as follows: cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, delphinidin-3-glucoside, delphinidin-3-galactoside, malvidin-3-glucoside, and malvidin-3-galactoside by Extrasynthese (Genay, France); peonidin-3-glucoside, peonidin-3-galactoside, peonidin-3-arabinoside, and petunidin-3-glucoside by Polyphenols Laboratories AS (Sandnes, Norway); and (+)-catechin, epicatechin, procyanidin B1, procyanidin B2, procyanidin A2, quercetin-3-galactoside, quercetin-3-glucoside, querectin-3-rutinoside, quercetin-3-rhamnoside, quercetin-3-glucuronide, quercetin, myricetin, keampferol-7-neohesperidoside, gallic acid, caffeic acid, *p*-coumaric acid, ferulic acid, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, 1,5-dicaffeoylquinic acid, esculetin, scopoletin, and phloridzin by Sigma-Aldrich (St. Louis, MO, USA).

LC-MS grade methanol and water were obtained from J.T. Baker (Deventer, the Netherlands). HPLC grade methanol and formic acid eluent additive for LC-MS were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium fluoride was obtained by Merck (Darmstadt, Germany). Ultrapure water was taken from a Milli-Q system supplied by Millipore (Billerica, MA, USA). Nylon membranes (porosity 0.2 μm) for the filtration of the bilberry extracts before HPLC analysis were obtained from VWR™ International (Radnor, PA, USA).

### Fruit sampling and postharvest treatment

*V. myrtillus* and *V. uliginosum* L. subsp. *gaultherioides* samples analyzed in the present study consisted of blends of berries collected in 15 different zones of Tuscan Apennines in August 2014 (see Table S1 of the Electronic supplementary material). Hence, representative samples of the two wild berry

species were obtained for the whole investigated area. In these samples, the variations in polyphenolic composition within the species, due to different genotypes and/or environmental conditions (such as altitude and solar exposure of collection areas), should be therefore minimized.

In order to confirm the attribution to *V. uliginosum* subsp. *gaultherioides* of the "false bilberry" plants included in the present study, a genetic analysis was carried out following the specifications reported by Ancillotti and coworkers [4].

The *V. corymbosum* sample was a mixture of berries of the genotypes "Duke," "Berkely," and "Bluecrop," cultivated in a site included in the area of Tuscan Apennines selected for the harvest of wild species. These cultivars were chosen on the basis of their wide diffusion in the Italian market [21].

After the sampling, all berries were immediately frozen in liquid nitrogen, freeze-dried, and finally ground in order to obtain a homogeneous powder. All samples were stored at −20 °C until analyses were performed.

### Sample extraction

Three representative aliquots from each berry sample were extracted according to a procedure previously developed for *Fragaria vesca* berries [22] and successively verified for bilberry and "false bilberry" [4]. Briefly, about 500 mg dry weight (d.w.) berry aliquots were homogenized in an ice bath under magnetic stirring with 15 mL of a methanol/water solution 8/2 (*v/v*), containing NaF 10 mM to inactivate polyphenol oxidase; the mixture was centrifuged at 1800×*g* for 5 min and the supernatant recovered. This procedure was repeated three times and the resulting extracts were combined. The organic solvent was removed by vacuum evaporation, acidified with formic acid up to pH = 2.0 ± 0.1 (volume of formic acid 170–190 μL), and filtrated at 0.2 μm with nylon membranes, before LC-MS/MS analysis. A final extract volume of approximately 9.2 mL was therefore obtained.

### LC-TOF and LC-Q/TOF analysis

LC analysis was performed on an Agilent Infinity 1290 system equipped with an Acquity BEH C18 column (15 cm × 2.1 cm i.d., particle size 1.7 μm) and a guard column containing the same stationary phase (Waters, Milford, MA, USA). Column temperature was set at 50 °C. Water/formic acid 95:5 *v/v* (eluent A) and methanol/formic acid 95:5 *v/v* (eluent B) were used for the analyte elution, according to the following gradients: 0–2 min isocratic 2% B, 2–30 min linear gradient 2–30% B, 30–35 min linear gradient 30–95% B, and 35–37 min isocratic 95% B. The flow rate was 450 μL/min and the injection volume was 2 μL.

The LC system was coupled with a SCIEX (Framingham, MA, USA) TripleTOF® 5600 hybrid Q/TOF mass analyzer by the DuoSpray™ Source for MS and MS/MS analysis and

the following source parameters were kept constant during the whole acquisition: heater temperature 400 °C, Curtain Gas™ 25, nebulizing gas 45, heating gas 45, and spray voltage +5300 and −4500 V for positive and negative polarity, respectively.

Each sample was analyzed, both under positive and negative ionization, using two different mass acquisition methods for each ionization mode. The first one consisted of a high-resolution TOF MS survey scan (from 100 to 2000 Da, cycle time 250 ms). The second acquisition method was a TOF survey scan experiment from 100 to 2000 Da (accumulation time 250 ms), followed by the selection of the top 10 candidate ions collected within each cycle, by the Information Dependent Acquisition (IDA) software. Q/TOF MS/MS spectra of the ions selected in each cycle were then acquired from 100 to 2000 Da, each one with an accumulation time of 75 ms, using a collision energy of 35 eV and a collision energy spread of ±15 eV (whole cycle time 1050 ms). In order to enhance the general quality of MS/MS spectra of peaks with low signal intensity, Q/TOF MS/MS analysis was also performed using narrower mass ranges, typically from 100 to 1250 Da (accumulation time of 50 ms and whole cycle time 800 ms).

Automated calibration was performed using an external calibrant delivery system (CDS) which infuses proper calibration solution prior to sample introduction.

### Data processing and metabolite identification

The high number of information derived from the 5600 TripleTOF® analysis of investigated samples, both in negative and positive ionization, needs to be processed with specific software. PeakView® 2.2 and MasterView® 1.1 software were used for the compound identification based on the TOF accurate mass and isotope pattern determinations, as well as on the Q/TOF fragmentation spectra of parent ions.

The following identification criteria were adopted in this study.

– TOF accuracy of the pseudo-molecular ion: <5 ppm
– Isotope ratio difference compared to the theoretical isotope profile: <20%
– Purity score of the MS/MS spectra compared to the one of available standards: ≥80%

In this manuscript, we used the words "identification/identified," sometimes stressed by the words "undoubted/undoubtedly," "unequivocal/unequivocally" when an authentic reference standard was available. Conversely, the terms "putative/putatively" or "tentative/tentatively" were used in the sentence when the reference standard was not available.

Then, in order to compare the polyphenolic compositions of the three investigated species and to highlight the polyphenols that mainly contributed to their differentiation, principal

component analysis (PCA) of molecular or quasi-molecular ions of identified and putatively assigned compounds was performed using MarkerView 1.2.1 software. This approach was carried out separately for negative and positive ionization modes. Quality control (QC) of PCA was performed, using a QC sample, consisting of a mixture of equal aliquots of each berry extract. QC evaluation was carried out by verifying if PCA object scores obtained by replicated injections of the QC sample were close to the origin of PCA coordinates.

## Results and discussion

### Compound identification by LC-ESI-TOF and LC-ESI-Q/TOF analysis

The polyphenols found in berries of the investigated *Vaccinium* species were identified according to their chromatographic behavior, their TOF MS and Q/TOF MS/MS spectra, also in comparison with standard reference compounds, when available. Both positive and negative ionization modes were used for compound attribution.

Molecules that were unequivocally or putatively identified belonged to the compound classes of anthocyanins, flavonols, flavanols, and phenolic acids; other polyphenolic compounds belonging to miscellaneous classes (e.g., coumarins and dihydrochalcones) were also tentatively recognized.

Compound identification within each class is detailed below and summarized in Tables 1, 2, 3, and 4, which show retention time (Rt, min), mass (Da) found by the TOF survey scan experiment and main MS/MS fragments (Da) obtained by the Q/TOF experiment, proposed formula and corresponding exact mass (Da), mass accuracy ($\Delta$, ppm), and putative identification of the peaks considered. Peaks reported in these tables were also shown in Figs. S1–S4 of the Electronic supplementary material (ESM).

#### Anthocyanins

Anthocyanins are characterized by a positive charge at pH < 3 and therefore are typically determined in the form of molecular ion $[M]^+$ [23]; accordingly, these polyphenols were identified under positive ionization (Table 1). Moreover, their attribution was also confirmed under negative ionization, by monitoring the quasi-molecular ion $[M-2H]^-$, according to the mass spectrometric behavior observed for these polyphenols by Sun and colleagues [24]. However, for this latter ionization mode, a less complete profile of the anthocyanin fraction was obtained, owing to its generally lower sensitivity that prevented in several cases the signal detection (data not shown).

It should also be remarked that when the anthocyanin has molecular weight 1 Da higher than that of a flavonol (i.e.,

delphinidin vs. quercetin, cyanidin vs. kaempferol, and petunidin vs. isorhamnetin derivatives of the same sugar), the $[M]^+$ or the $[M-2H]^-$ ions of the former and the $[M+H]^+$ or the $[M-H]^-$ ions of the latter are isobars, thus making relevant for their discrimination the chromatographic behavior.

As widely reported elsewhere [15, 18, 25], also in this study, MS/MS fragmentation of anthocyanins produced only the loss of the sugar units (e.g., 162 Da for a hexose and 132 Da for a pentose) and the corresponding detection of the aglycone fragment (i.e., 287.06 Da for cyanidin, 303.05 Da for delphinidin, 317.07 Da for petunidin, 301.07 Da for peonidin, and 331.08 Da for malvidin) (Table 1).

Using the IDA TOF-Q/TOF workflow and, when possible, by comparing the retention time and mass spectra of unknowns with those of authentic standards, the undoubted or at least the tentative identification of 64 anthocyanins was achieved. TOF MS $[M]^+$ molecular ions matched the proposed formulae with very high mass accuracy, being $\Delta$ absolute values ≤1 ppm in about 80% of the cases, and included in the range of 1.1–2.2 ppm for the remaining compounds (Table 1). Among the 64 anthocyanins identified, the presence of the 3-*O*-glucoside derivatives of delphinidin, cyanidin, petunidin, peonidin, and malvidin (peaks 9, 16, 22, 28, and 34); 3-*O*-galactoside derivatives of delphinidin, cyanidin, peonidin, and malvidin (peaks 6, 12, 25, and 29); and 3-*O*-arabinoside derivatives of cyanidin and peonidin (peaks 18 and 32) was confirmed by spiking the extracts with authentic reference standards (Table 1). Peaks 11, 17, 26, and 36 were putatively annotated to delphinidin-3-*O*-arabinoside, petunidin-3-*O*-galactoside, petunidin-3-*O*-arabinoside, and malvidin-3-*O*-arabinoside on the basis of their (i) TOF MS accuracy, isotope ratio difference, and MS/MS data ($Y_0$ cleavage of the sugar and formation of the aglycone ion), as well as (ii) relative chromatographic retention, the latter being in agreement with the retention observed by various authors under reversed-phase conditions for different glycosides with the same aglycone (i.e., increasing retention in the order galactoside < glucoside < arabinoside) and for different anthocyanins glycosylated with the same sugar (i.e., increasing retention in the order delphinidin < cyanidin < petunidin < peonidin < malvidin) [4, 7, 23]. The 15 anthocyanins reported above were detected in all the investigated species and resulted in all cases among the most abundant anthocyanidin derivatives (signal intensities approximately included in between $1 \times 10^5$ and $1 \times 10^6$ counts), as widely reported elsewhere [4, 7, 8, 26].

Among the first eluting analytes (peaks 1–5, Rt = 9.7–11.4 min), which were detected only in the *V. myrtillus* berry extracts and with very low signal intensity (i.e., 1000–1800 counts), peaks 1, 3, and 5 exhibited an $[M]^+$ ion at 627.15 Da

t1.1 **Table 1** Retention times (Rt, min), [M]$^+$ molecular ions (TOF MS, Da), mass fragments (Q/TOF MS/MS, Da), proposed formula, corresponding exact mass (Da), and accuracy ($\Delta$, ppm) of peaks tentatively identified as anthocyanins in *V. myrtillus* (M), *V. uliginosum* L. subsp. *gaultherioides* (G), and *V. corymbosum* (C) under positive ionization. Symbols "+" and "−" mean detected and not detected

| | Peak | Rt | TOF MS | Q/TOF MS/MS | Proposed formula | Exact mass | $\Delta$ | M | G | C | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t1.3 | 1 | 9.7 | 627.1565 | 465.1078; 303.0485 | $C_{27}H_{31}O_{17}$ | 627.1556 | 1.4 | + | − | − | Delphinidin-dihexoside (I)[a] |
| t1.4 | 2 | 10.2 | 611.1677 | 449.1052; 287.0551 | $C_{27}H_{31}O_{16}$ | 611.1607 | 0.9 | + | − | − | Cyanidin-dihexoside (I)[a] |
| t1.5 | 3 | 10.5 | 627.1549 | 465.1078; 303.0485 | $C_{27}H_{31}O_{17}$ | 627.1556 | −1.0 | + | − | − | Delphinidin-dihexoside (II)[a] |
| t1.6 | 4 | 11.0 | 611.1609 | 449.1118; 287.0559 | $C_{27}H_{31}O_{16}$ | 611.1607 | 0.3 | + | − | − | Cyanidin-dihexoside (II)[a] |
| t1.7 | 5 | 11.4 | 627.1551 | 465.1007; 303.0502 | $C_{27}H_{31}O_{17}$ | 627.1556 | −0.8 | + | − | − | Delphinidin-dihexoside (III)[a] |
| t1.8 | 6 | 12.6 | 465.1031 | 303.0501 | $C_{21}H_{21}O_{12}$ | 465.1027 | 0.8 | + | + | + | Delphinidin-3-*O*-galactoside[b] |
| t1.9 | 7 | 13.0 | 479.0818 | 303.0495 | $C_{21}H_{19}O_{13}$ | 479.0820 | −0.1 | + | − | − | Delphinidin-glucuronide |
| t1.10 | 8 | 13.2 | 597.1447 | 303.0499 | $C_{26}H_{29}O_{16}$ | 597.1450 | −0.6 | + | − | − | Delphinidin-aldopentose-hexoside (I)[c] |
| t1.11 | 9 | 13.8 | 465.1032 | 303.0510 | $C_{21}H_{21}O_{12}$ | 465.1027 | 0.9 | + | + | + | Delphinidin-3-*O*-glucoside[b] |
| t1.12 | 10 | 14.0 | 597.1447 | 303.0502 | $C_{26}H_{29}O_{16}$ | 597.1450 | −0.6 | + | − | − | Delphinidin-aldopentose-hexoside (II)[c] |
| t1.13 | 11 | 14.8 | 435.0926 | 303.0505 | $C_{20}H_{19}O_{11}$ | 435.0922 | 0.9 | + | + | + | Delphinidin-3-*O*-arabinoside |
| t1.14 | 12 | 14.8 | 449.1081 | 287.0554 | $C_{21}H_{21}O_{11}$ | 449.1078 | 0.7 | + | + | + | Cyanidin-3-*O*-galactoside[b] |
| t1.15 | 13 | 15.4 | 611.1598 | 287.0551 | $C_{27}H_{31}O_{16}$ | 611.1607 | −1.5 | + | − | − | Cyanidin-dihexoside (III)[c] |
| t1.16 | 14 | 15.7 | 581.1502 | 287.0551 | $C_{26}H_{29}O_{15}$ | 581.1501 | 0.3 | + | − | − | Cyanidin-aldopentose-hexoside (I)[c] |
| t1.17 | 15 | 15.8 | 463.0867 | 287.0543 | $C_{21}H_{19}O_{12}$ | 463.0871 | −0.9 | + | − | − | Cyanidin-glucuronide |
| t1.18 | 16 | 16.3 | 449.1080 | 287.0555 | $C_{21}H_{21}O_{11}$ | 449.1078 | 0.3 | + | + | + | Cyanidin-3-*O*-glucoside[b] |
| t1.19 | 17 | 16.9 | 479.1188 | 317.0659 | $C_{22}H_{23}O_{12}$ | 479.1184 | 0.8 | + | + | + | Petunidin-3-*O*-galactoside |
| t1.20 | 18 | 17.0 | 419.0976 | 287.0558 | $C_{20}H_{19}O_{10}$ | 419.0978 | 0.9 | + | + | + | Cyanidin-3-*O*-arabinoside[b] |
| t1.21 | 19 | 17.2 | 581.1503 | 287.0555 | $C_{26}H_{29}O_{15}$ | 581.1501 | 0.3 | + | + | + | Cyanidin-aldopentose-hexoside (II)[c] |
| t1.22 | 20 | 17.6 | 493.0973 | 317.0652 | $C_{22}H_{21}O_{13}$ | 493.0977 | −0.8 | + | − | − | Petunidin-glucuronide |
| t1.23 | 21 | 17.9 | 611.1603 | 317.0650 | $C_{27}H_{31}O_{16}$ | 611.1607 | −0.6 | + | − | − | Petunidin-aldopentose-hexoside[c] |
| t1.24 | 22 | 18.1 | 479.1188 | 317.0657 | $C_{22}H_{23}O_{12}$ | 479.1184 | 0.9 | + | + | + | Petunidin-3-*O*-glucoside[b] |
| t1.25 | 23 | 18.6 | 551.1392 | 287.0546 | $C_{25}H_{27}O_{14}$ | 551.1395 | −0.5 | + | − | − | Cyanidin-aldodipentoside[c] |
| t1.26 | 24 | 18.7 | 435.0932 | 303.0492 | $C_{20}H_{19}O_{11}$ | 435.0922 | 2.2 | + | + | + | Delphinidin-3-*O*-xyloside |
| t1.27 | 25 | 18.9 | 463.1235 | 301.0713 | $C_{22}H_{23}O_{11}$ | 463.1235 | 0.1 | + | + | + | Peonidin-3-*O*-galactoside[b] |
| t1.28 | 26 | 19.0 | 449.1081 | 317.0660 | $C_{21}H_{21}O_{11}$ | 449.1078 | 0.5 | + | + | + | Petunidin-3-*O*-arabinoside |
| t1.29 | 27 | 19.6 | 595.1652 | 301.0715 | $C_{27}H_{31}O_{15}$ | 595.1657 | −0.9 | + | − | − | Peonidin-aldopentose-hexoside (I)[c] |
| t1.30 | 28 | 20.2 | 463.1238 | 301.0714 | $C_{22}H_{23}O_{11}$ | 463.1235 | 0.7 | + | + | + | Peonidin-3-*O*-glucoside[b] |
| t1.31 | 29 | 20.3 | 493.1342 | 331.0816 | $C_{23}H_{25}O_{12}$ | 493.1340 | 0.4 | + | + | + | Malvidin-3-*O*-galactoside[b] |
| t1.32 | 30 | 20.5 | 419.0971 | 287.0552 | $C_{20}H_{19}O_{10}$ | 419.0973 | −0.4 | + | + | + | Cyanidin-aldopentoside |
| t1.33 | 31 | 20.8 | 595.1661 | 301.0708 | $C_{27}H_{31}O_{15}$ | 595.1657 | 0.6 | + | − | − | Peonidin-aldopentose-hexoside (II)[c] |
| t1.34 | 32 | 21.0 | 433.1131 | 301.0711 | $C_{21}H_{21}O_{10}$ | 433.1129 | 0.5 | + | + | + | Peonidin-3-*O*-arabinoside[b] |
| t1.35 | 33 | 21.1 | 507.1137 | 303.0502 | $C_{23}H_{23}O_{13}$ | 507.1133 | 0.7 | − | − | + | Delphinidin-acetyl-hexoside (I) |
| t1.36 | 34 | 21.3 | 493.1345 | 331.0818 | $C_{23}H_{25}O_{12}$ | 493.1340 | 0.8 | + | + | + | Malvidin-3-*O*-glucoside[b] |
| t1.37 | 35 | 21.4 | 419.0976 | 287.0540 | $C_{20}H_{19}O_{10}$ | 419.0973 | 0.7 | + | + | + | Cyanidin-3-*O*-xyloside |
| t1.38 | 36 | 22.3 | 463.1237 | 331.0822 | $C_{22}H_{23}O_{11}$ | 463.1235 | 0.4 | + | + | + | Malvidin-3-*O*-arabinoside |
| t1.39 | 37 | 23.1 | 449.1086 | 317.0659 | $C_{21}H_{21}O_{11}$ | 449.1078 | 1.8 | + | + | + | Petunidin-3-*O*-xyloside |
| t1.40 | 38 | 23.2 | 639.1924 | 331.0807 | $C_{29}H_{35}O_{16}$ | 639.1919 | 0.7 | − | − | + | Malvidin-deoxyhexose-hexoside |
| t1.41 | 39 | 23.5 | 535.1076 | 287.0556 | $C_{24}H_{23}O_{14}$ | 535.1082 | −1.1 | − | − | + | Cyanidin-malonyl-hexoside |
| t1.42 | 40 | 23.7 | 491.1186 | 287.0550 | $C_{23}H_{23}O_{12}$ | 491.1184 | 0.4 | + | − | + | Cyanidin-acetyl-hexoside (I) |
| t1.43 | 41 | 24.5 | 507.1136 | 303.0405 | $C_{23}H_{23}O_{13}$ | 507.1133 | 0.6 | − | − | + | Delphinidin-acetyl-hexoside (II) |
| t1.44 | 42 | 25.2 | 579.1339 | 331.0819 | $C_{26}H_{27}O_{15}$ | 579.1344 | −0.9 | − | − | + | Malvidin-malonyl-hexoside (I) |
| t1.45 | 43 | 25.4 | 521.1290 | 317.0645 | $C_{24}H_{25}O_{13}$ | 521.1290 | 0.1 | + | − | + | Petunidin-acetyl-hexoside (I) |
| t1.46 | 44 | 25.4 | 433.1138 | 301.0714 | $C_{21}H_{21}O_{10}$ | 433.1129 | 2.1 | + | + | + | Peonidin-3-*O*-xyloside |
| t1.47 | 45 | 26.2 | 463.1240 | 331.0820 | $C_{22}H_{23}O_{11}$ | 463.1235 | 1.2 | + | + | + | Malvidin-3-*O*-xyloside |
| t1.48 | 46 | 27.2 | 505.1343 | 301.0703 | $C_{24}H_{25}O_{12}$ | 505.1340 | 0.5 | + | − | + | Peonidin-acetyl-hexoside (I) |
| t1.49 | 47 | 27.2 | 491.1189 | 287.0552 | $C_{23}H_{23}O_{12}$ | 491.1184 | 1.0 | + | − | + | Cyanidin-acetyl-hexoside (II) |

t1.50 **Table 1** (continued)

| Peak | Rt | TOF MS | Q/TOF MS/MS | Proposed formula | Exact mass | $\Delta$ | M | G | C | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|---|
| t1.51 48 | 27.5 | 579.1349 | 331.0825 | $C_{26}H_{27}O_{15}$ | 579.1344 | 0.9 | − | − | + | Malvidin-malonyl-hexoside (II) |
| t1.52 49 | 27.9 | 535.1455 | 331.0813 | $C_{25}H_{27}O_{13}$ | 535.1446 | 1.7 | + | − | + | Malvidin-acetyl-hexoside (I) |
| t1.53 50 | 28.2 | 521.1293 | 317.0662 | $C_{24}H_{25}O_{13}$ | 521.1290 | 0.7 | + | − | + | Petunidin-acetyl-hexoside (II) |
| t1.54 51 | 29.3 | 611.1401 | 303.0503 | $C_{30}H_{27}O_{14}$ | 611.1395 | 1.0 | + | − | − | Delphinidin-coumaroyl-hexoside (I) |
| t1.55 52 | 30.5 | 505.1345 | 301.0702 | $C_{24}H_{25}O_{12}$ | 505.1340 | 0.8 | + | − | + | Peonidin-acetyl-hexoside (II) |
| t1.56 53 | 30.7 | 535.1456 | 331.0814 | $C_{25}H_{27}O_{13}$ | 535.1446 | 1.9 | + | − | + | Malvidin-acetyl-hexoside (II) |
| t1.57 54 | 31.3 | 595.1451 | 287.0549 | $C_{30}H_{27}O_{13}$ | 595.1446 | 0.8 | + | + | − | Cyanidin-coumaroyl-hexoside (I) |
| t1.58 55 | 31.5 | 611.1395 | 303.0496 | $C_{30}H_{27}O_{14}$ | 611.1395 | 0.0 | + | − | − | Delphinidin-coumaroyl-hexoside (II) |
| t1.59 56 | 31.7 | 625.1551 | 317.0652 | $C_{31}H_{29}O_{14}$ | 625.1552 | −0.1 | + | − | + | Petunidin-coumaroyl-hexoside (I) |
| t1.60 57 | 32.1 | 595.1457 | 287.0556 | $C_{30}H_{27}O_{13}$ | 595.1446 | 1.9 | + | + | − | Cyanidin-coumaroyl-hexoside (II) |
| t1.61 58 | 32.1 | 609.1609 | 301.0712 | $C_{31}H_{29}O_{13}$ | 609.1603 | 1.1 | + | − | − | Peonidin-coumaroyl-hexoside (I) |
| t1.62 59 | 32.2 | 625.1557 | 317.0656 | $C_{31}H_{29}O_{14}$ | 625.1552 | 0.8 | + | − | + | Petunidin-coumaroyl-hexoside (II) |
| t1.63 60 | 32.2 | 639.1717 | 331.0811 | $C_{32}H_{31}O_{14}$ | 639.1708 | 1.4 | + | − | + | Malvidin-coumaroyl-hexoside (I) |
| t1.64 61 | 32.3 | 669.1821 | 331.0812 | $C_{33}H_{33}O_{15}$ | 669.1814 | 1.0 | + | − | − | Malvidin-feruloyl-hexoside (I) |
| t1.65 62 | 32.4 | 609.1611 | 301.0712 | $C_{31}H_{29}O_{13}$ | 609.1603 | 1.3 | + | − | − | Peonidin-coumaroyl-hexoside (II) |
| t1.66 63 | 32.4 | 639.1719 | 331.0824 | $C_{32}H_{31}O_{14}$ | 639.1708 | 1.8 | + | + | + | Malvidin-coumaroyl-hexoside (II) |
| t1.67 64 | 32.5 | 669.1819 | 331.0810 | $C_{33}H_{33}O_{15}$ | 669.1814 | 0.8 | + | − | − | Malvidin-feruloyl-hexoside (II) |

[a] Hexoses separately linked to the aglycone

[b] Confirmed by spiking the extracts with authentic standards

[c] Hexoses linked as disaccharides

366 and a fragmentation pattern with ions at $m/z$ 465.10 (loss of
367 162 Da, hexose residue) and $m/z$ 303.05 (further loss of a
368 hexose unit), the latter corresponding to the delphinidin agly-
369 cone. Similarly, peaks 2 and 4 ([M]$^+$ ion at $m/z$ 611.16) gave
370 rise to two independent losses of 162 Da, producing the frag-
371 ments at $m/z$ 449.11 (i.e., cyanidin monoglycoside) and $m/z$
372 287.06 (i.e., cyanidin). In order to correctly interpret these
373 findings, it should be noted that the ESI-MS/MS fragmenta-
374 tion of anthocyanin derivatives with two sugars linked to dif-
375 ferent hydroxyls of the aglycone actually produces the agly-
376 cone monoglycoside as a consequence of the $Y_0$ cleavage of
377 one sugar [27]. Conversely, in the case of disaccharide deriv-
378 atives of anthocyanins, only the molecular ion and the agly-
379 cone fragment have been reported [28]. On the basis of the
380 aforementioned considerations, peaks 1–5 can be putatively
381 ascribed to dihexoside derivatives of delphinidin and
382 cyanidin, the two hexoses being separately linked to the agly-
383 cone. Remarkably, only one anthocyanidin derivative with
384 two hexose units linked in different aglycone positions (i.e.,
385 cyanidin) was elsewhere reported [25].

386 Peak 13 (Rt = 15.4 min, signal intensity 1200 counts)
387 showed a [M]$^+$ at $m/z$ 611.16 and only one fragment at $m/z$
388 287.05. Accordingly, it was tentatively assigned to a cyanidin
389 hexose-hexose disaccharide. In this regard, it should be noted
390 that such an attribution is in agreement with the reversed-
391 phase chromatographic behavior of anthocyanidins
392 diglycosides, since, for example, 3,5-diglycoside derivatives

393 have been reported to elute before the corresponding 3-
394 diglycosides [23, 29]. Interestingly, the occurrence of cyanidin
395 disaccharides was not previously reported in bilberry.

396 Peaks 7, 15, and 20, which had moderate intensity (i.e.,
397 4000–11,000 counts) were peculiar of the *V. myrtillus* extract.
398 These peaks were ascribed to glucuronide derivatives of
399 delphinidin, cyanidin, and petunidin, respectively. In fact,
400 peaks 7, 15, and 20 were characterized by the loss in common
401 of 176 Da, consistent with glucopyranuronic acid, and the
402 consequent formation of fragments at $m/z$ 303.05,
403 287.05, and $m/z$ 317.07, respectively, attributable to
404 delphinidin, cyanidin, and petunidin. Relative chromato-
405 graphic retention of these peaks in respect to the correspond-
406 ing glucoside derivatives was also in accordance with their
407 putative identification as glucuronide derivatives [30, 31]. It
408 should be underlined that this study is the first one reporting
409 the identification of delphinidin, cyanidin, and petunidin glu-
410 curonides in *V. myrtillus* fruits.

411 Peaks 8, 10, 14, 19, 21, 27, and 31, which were detected
412 only in the *V. myrtillus* extract, exhibited the communal loss of
413 294 Da, attributable to an aldopentose-hexose residue, and
414 MS/MS resulting fragments at $m/z$ 303.05 (delphinidin), $m/z$
415 287.05 (cyanidin), $m/z$ 317.07 (petunidin), and $m/z$ 301.07
416 (peonidin). In this regard, it is notable that the chromatograph-
417 ic retention order of the aforementioned peaks was in agree-
418 ment with the proposed aglycone attribution. It should also be
419 noted that peaks 8 (i.e., delphinidin disaccharide), 14 (i.e.,

Q2 t2.1 **Table 2** Retention times (Rt, min), [M−H]⁻ quasi-molecular ions (TOF MS, Da), main mass fragments (Q/TOF MS/MS, Da), proposed formula, corresponding exact mass (Da), and accuracy (Δ, ppm) of peaks tentatively identified as flavonols in *V. myrtillus* (M), *V. uliginosum* L. subsp. *gaultherioides* (G), and *V. corymbosum* (C) under negative ionization. Mass fragments in italics refer to the most intense signals. Symbols "+" and "−" mean detected and not detected

| | Peak | Rt | TOF MS | Q/TOF MS/MS | Proposed formula | Exact mass | Δ | M | G | C | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t2.3 | 65 | 19.6 | 493.0617 | *317.0297*; 178.9989; 151.0036 | $C_{21}H_{18}O_{14}$ | 493.0624 | −1.3 | + | + | + | Myricetin-glucuronide |
| t2.4 | 66 | 19.9 | 479.0826 | 317.0299; *316.0225*; 271.0229 | $C_{21}H_{20}O_{13}$ | 479.0831 | −1.1 | + | + | + | Myricetin-3-*O*-galactoside |
| t2.5 | 67 | 20.6 | 479.0830 | 317.0299; *316.0216*; 271.0236 | $C_{21}H_{20}O_{13}$ | 479.0831 | −0.1 | + | + | + | Myricetin-3-*O*-glucoside |
| t2.6 | 68 | 20.9 | 449.0713 | 317.0288; *316.0207*; 271.0236 | $C_{20}H_{18}O_{12}$ | 449.0725 | −2.8 | + | + | − | Myricetin-3-*O*-aldopentoside (I) |
| t2.7 | 69 | 22.5 | 449.0714 | 317.0281; *316.0212*; 271.0230 | $C_{20}H_{18}O_{12}$ | 449.0725 | −2.6 | − | + | + | Myricetin-3-*O*-aldopentoside (II) |
| t2.8 | 70 | 22.9 | 449.0715 | 317.0240; *316.0211*; 271.0251 | $C_{20}H_{18}O_{12}$ | 449.0725 | −2.4 | + | + | + | Myricetin-3-*O*-aldopentoside (III) |
| t2.9 | 71 | 24.1 | 463.0876 | 301.0346; *300.0271*; 271.0248 | $C_{21}H_{20}O_{12}$ | 463.0882 | −1.4 | + | + | + | Quercetin-3-*O*-galactoside[a] |
| t2.10 | 72 | 24.2 | 521.0926 | 317.0278; *316.0216*; 271.0246 | $C_{23}H_{22}O_{14}$ | 521.0937 | −2.1 | − | − | + | Myricetin-acetyl-hexoside (I) |
| t2.11 | 73 | 24.4 | 477.0661 | *301.0352*; 178.9979; 151.0028 | $C_{21}H_{18}O_{13}$ | 477.0675 | −2.8 | + | + | + | Quercetin-glucuronide[a] |
| t2.12 | 74 | 24.6 | 609.1452 | 301.0335; *300.0262*; 271.0235 | $C_{27}H_{30}O_{16}$ | 609.1461 | −1.5 | + | − | + | Quercetin-3-*O*-deoxyhexose-hexoside |
| t2.13 | 75 | 25.0 | 463.0879 | 301.0346; *300.0267*; 271.0248 | $C_{21}H_{20}O_{12}$ | 463.0882 | −0.7 | + | + | + | Quercetin-3-*O*-glucoside[a] |
| t2.14 | 76 | 25.6 | 609.1454 | 301.0335; *300.0255* | $C_{27}H_{30}O_{16}$ | 609.1461 | −1.2 | − | − | + | Quercetin-3-*O*-rutinoside[a] |
| t2.15 | 77 | 25.7 | 433.0762 | 301.0341; *300.2630*; 271.0230 | $C_{20}H_{18}O_{11}$ | 433.0776 | −3.3 | + | + | + | Quercetin-3-*O*-aldopentoside (I) |
| t2.16 | 78 | 26.1 | 493.0983 | 331.0450; *330.0369*; 315.1320 | $C_{22}H_{22}O_{13}$ | 493.0988 | −1.0 | + | + | + | Laricitrin-3-*O*-galactoside |
| t2.17 | 79 | 26.4 | 433.0768 | 301.0341; *300.0267*; 271.0237 | $C_{20}H_{18}O_{11}$ | 433.0776 | −1.9 | + | + | + | Quercetin-3-*O*-aldopentoside (II) |
| t2.18 | 80 | 26.4 | 521.0941 | 317.0271; *316.0202*; 271.0225 | $C_{23}H_{22}O_{14}$ | 521.0937 | 0.7 | − | − | + | Myricetin-acetyl-hexoside (II) |
| t2.19 | 81 | 26.5 | 317.0294 | 178.9983; 165.0192; *151.0031*; 137.0237 | $C_{15}H_{10}O_{8}$ | 317.0303 | −2.8 | + | + | + | Myricetin[a] |
| t2.20 | 82 | 26.6 | 493.0989 | 331.0461; *330.0383*; 315.0151 | $C_{22}H_{22}O_{13}$ | 493.0988 | 0.3 | + | + | + | Laricitrin-3-*O*-glucoside |
| t2.21 | 83 | 26.7 | 507.0777 | *331.0445*; 316.0210; 178.9978 | $C_{22}H_{20}O_{14}$ | 507.0780 | −0.7 | + | + | + | Laricitrin-glucuronide |
| t2.22 | 84 | 26.7 | 549.0876 | 505.0983; 463.0870; 301.0333; *300.0263* | $C_{24}H_{22}O_{15}$ | 549.0886 | −1.8 | − | − | + | Quercetin-malonyl-hexoside (I) |
| t2.23 | 85 | 27.0 | 549.0877 | 505.0997; 463.0892; 301.0350; *300.0271* | $C_{24}H_{22}O_{15}$ | 549.0886 | −1.6 | − | − | + | Quercetin-malonyl-hexoside (II) |
| t2.24 | 86 | 27.2 | 447.0912 | 285.0395; *284.0308*; 255.0280; 227.0335 | $C_{21}H_{20}O_{11}$ | 447.0933 | −3.2 | + | + | + | Kaempferol-3-*O*-galactoside |
| t2.25 | 87 | 27.5 | 433.0768 | 301.0343; *300.0270*; 271.0236 | $C_{20}H_{18}O_{11}$ | 433.0776 | −2.0 | − | + | + | Quercetin-3-*O*-aldopentoside (III) |
| t2.26 | 88 | 28.0 | 463.0862 | 331.0456; *330.0361*; 315.0149 | $C_{21}H_{20}O_{12}$ | 463.0882 | −4.4 | + | + | + | Laricitrin-3-*O*-xyloside |
| t2.27 | 89 | 28.2 | 505.0972 | 301.0338; *300.0273*; 271.0237 | $C_{23}H_{22}O_{13}$ | 505.0988 | −3.0 | − | − | + | Quercetin-acetyl-hexoside (I) |
| t2.28 | 90 | 28.4 | 461.0721 | *285.0390*; 257.0438; 229.0491 | $C_{21}H_{18}O_{12}$ | 461.0725 | −0.9 | + | + | + | Kaempferol-glucuronide |
| t2.29 | 91 | 28.5 | 463.0869 | 331.0431; *330.0378*; 315.0140 | $C_{21}H_{20}O_{12}$ | 463.0882 | −2.9 | + | + | + | Laricitrin-3-*O*-aldopentoside (I) |
| t2.30 | 92 | 28.8 | 447.0923 | 301.0350; *300.0271*; 271.0238 | $C_{21}H_{20}O_{11}$ | 447.0933 | −2.1 | + | − | + | Quercetin-3-*O*-rhamnoside[a] |
| t2.31 | 93 | 28.9 | 447.0923 | 285.0397; *284.0325*; 255.0293; 227.0348 | $C_{21}H_{20}O_{11}$ | 447.0933 | −2.1 | − | + | − | Kaempferol-3-*O*-glucoside |
| t2.32 | 94 | 29.3 | 417.0823 | *284.0316*; 255.0291; 227.0343 | $C_{20}H_{18}O_{10}$ | 417.0827 | −0.9 | + | + | + | Kaempferol-3-*O*-aldopentoside |
| t2.33 | 95 | 29.3 | 505.0979 | 301.0352; *300.0272*; 271.0237 | $C_{23}H_{22}O_{13}$ | 505.0988 | −1.6 | − | − | + | Quercetin-acetyl-hexoside (II) |
| t2.34 | 96 | 29.5 | 593.1504 | *285.0398*; 284.0326 | $C_{27}H_{30}O_{15}$ | 593.1512 | −1.3 | − | − | + | Kaempferol-7-*O*-neohesperidoside[a] |
| t2.35 | 97 | 29.6 | 477.1032 | *314.0419*; 271.0232; 243.0290 | $C_{22}H_{22}O_{12}$ | 477.1038 | −1.4 | + | + | + | Isorhamnetin-3-*O*-galactoside |
| t2.36 | 98 | 29.7 | 505.0977 | 301.0337; *300.0268*; 255.0293 | $C_{23}H_{22}O_{13}$ | 505.0988 | −2.1 | − | − | + | Quercetin-acetyl-hexoside (III) |
| t2.37 | 99 | 30.0 | 463.0870 | *330.0355*; 331.0455; 315.0133 | $C_{21}H_{20}O_{12}$ | 463.0882 | −2.5 | + | + | + | Laricitrin-3-*O*-aldopentoside (II) |
| t2.38 | 100 | 30.0 | 579.1350 | 301.0351; *300.0262*; 271.0235 | $C_{26}H_{28}O_{15}$ | 579.1355 | −0.9 | + | − | − | Quercetin-3-*O*-deoxyhexose-pentoside |
| t2.39 | 101 | 30.2 | 623.1613 | 315.0496; *314.0405*; 299.0174 | $C_{28}H_{32}O_{16}$ | 623.1618 | −0.8 | − | − | + | Isorhamnetin-3-*O*-deoxyhexose-hexoside |
| t2.40 | 102 | 30.2 | 505.0983 | 301.0335; *300.0255*; 271.0226 | $C_{23}H_{22}O_{13}$ | 505.0988 | −0.9 | − | − | + | Quercetin-acetyl-hexoside (IV) |
| t2.41 | 103 | 30.4 | 477.1029 | *314.0426*; 271.0258; 243.0299 | $C_{22}H_{22}O_{12}$ | 477.1038 | −2.0 | + | + | − | Isorhamnetin-3-*O*-glucoside |
| t2.42 | 104 | 30.4 | 477.1029 | *331.0438*; 330.0362; 315.0135 | $C_{22}H_{22}O_{12}$ | 477.1038 | −2.0 | − | − | + | Laricitrin-3-*O*-rhamnoside |
| t2.43 | 105 | 30.6 | 535.1078 | *330.0362*; 315.097 | $C_{24}H_{24}O_{14}$ | 535.1093 | −2.9 | − | − | + | Laricitrin-acetyl-hexoside |
| t2.44 | 106 | 30.7 | 491.0833 | *315.0509*; 300.0255; 271.0239 | $C_{22}H_{20}O_{13}$ | 491.0831 | 0.4 | + | + | + | Isorhamnetin-glucuronide |
| t2.45 | 107 | 30.8 | 507.1141 | 345.0616; *344.0530*; 301.0353 | $C_{23}H_{24}O_{13}$ | 507.1144 | −0.7 | + | + | + | Syringetin-3-*O*-galactoside |

t2.46 **Table 2** (continued)

| Peak | Rt | TOF MS | Q/TOF MS/MS | Proposed formula | Exact mass | $\Delta$ | M | G | C | Tentative identification |
|------|----|--------|-------------|------------------|------------|----------|---|---|---|--------------------------|
| t2.47 108 | 30.9 | 623.1611 | *315.0492*; 314.0419; 299.0203 | $C_{28}H_{32}O_{16}$ | 623.1618 | −1.0 | − | − | + | Isorhamnetin-7-*O*-deoxyhexose-hexoside |
| t2.48 109 | 31.3 | 507.1147 | 345.0609; *344.0533*; 301.0343 | $C_{23}H_{24}O_{13}$ | 507.1144 | 0.6 | + | + | + | Syringetin-3-*O*-glucoside |
| t2.49 110 | 31.4 | 417.0814 | 285.0405; *284.0323*; 255.0299 | $C_{20}H_{18}O_{10}$ | 417.0827 | −3.1 | − | − | + | Kaempferol-3-*O*-aldopentoside |
| t2.50 111 | 31.5 | 521.0932 | *345.0600*; 330.0363; 315.0129 | $C_{23}H_{22}O_{14}$ | 521.0937 | 1.9 | + | + | + | Syringetin-glucuronide |
| t2.51 112 | 31.6 | 447.0931 | *314.0426*; 271.0238; 243.0289 | $C_{21}H_{20}O_{11}$ | 447.0933 | −0.3 | + | + | + | Isorhamnetin-3-*O*-aldopentoside (I) |
| t2.52 113 | 31.8 | 447.0919 | *314.0413*; 299.0251 | $C_{21}H_{20}O_{11}$ | 447.0933 | −3.2 | + | + | + | Isorhamnetin-3-*O*-aldopentoside (II) |
| t2.53 114 | 32.0 | 477.1025 | *344.0544*; 301.0341; 273.0390 | $C_{22}H_{22}O_{12}$ | 477.1038 | −2.8 | + | + | + | Syringetin-3-*O*-aldopentoside (I) |
| t2.54 115 | 32.1 | 301.0347 | 178.9980; *151.0033*; 149.0219; 121.0282 | $C_{15}H_{10}O_7$ | 301.0354 | −2.4 | + | + | + | Quercetin[a] |
| t2.55 116 | 32.2 | 447.0920 | *314.0411*; 271.0218; 243.0283 | $C_{21}H_{20}O_{11}$ | 447.0933 | −2.8 | − | + | + | Isorhamnetin-3-*O*-aldopentoside (III) |
| t2.56 117 | 32.2 | 477.1026 | *344.0537*; 301.0354; 273.0382 | $C_{22}H_{22}O_{12}$ | 477.1038 | −2.5 | + | + | + | Syringetin-3-*O*-aldopentoside (II) |
| t2.57 118 | 32.6 | 331.0458 | *316.0196*; 178.9976; 151.0062 | $C_{16}H_{12}O_8$ | 331.0459 | −0.4 | + | + | + | Laricitrin |
| t2.58 119 | 33.3 | 315.0504 | *300.0269*; 271.0227; 151.0026 | $C_{16}H_{12}O_7$ | 315.0510 | −1.9 | + | + | + | Isorhamnetin |

[a] Confirmed by spiking the extracts with authentic standards

420 cyanidin disaccharide), 21 (i.e., petunidin disaccharide), and
421 27 (peonidin disaccharide) eluted between galactoside and
422 glucoside derivatives of the corresponding aglycone, thus sug-
423 gesting their putative identification as sambubioside deriva-
424 tives [23]. This hypothesis is in agreement with the findings of
425 Du and coworkers, who reported the occurrence of
426 delphinidin-3-sambubioside and cyanidin-3-sambubioside in
427 bilberry [32].

428 Peak 23 showed a neutral loss of 264 Da, corresponding to
429 an aldopentose disaccharide, and the resulting formation of a
430 fragment at *m/z* 287.05 (i.e., cyanidin). This peak was found
431 only in *V. myrtillus* extract, in agreement with the results pre-
432 viously reported by Latti and coworkers [25].

433 Peaks 24, 30, 35, 37, 44, and 45 detected in berries
434 from all the investigated *Vaccinium* species (Table 1) and can
435 be ascribed to aldopentoside derivatives of delphinidin (peak
436 24), cyanidin (peaks 30 and 35), petunidin (peak 37), peonidin
437 (peak 44), and malvidin (peak 45), based on the neutral loss of
438 132 Da (i.e., aldopentose) and the formation of the corre-
439 sponding aglycone fragment. These peaks occurred in the
440 three investigated berry species with very different intensities,
441 being those determined in *V. uliginosum* subsp. *gaultherioides*
442 characterized by much higher signals than the others. It should
443 also be underlined that peak 30 differentiate itself from the
444 others, owing to a much lower signal intensity. It is remarkable
445 that this study is the first one putatively identifying
446 anthocyanidin aldopentosides in blueberry and aldopentose
447 derivatives of delphinidin, cyanidin, and peonidin in bilberry,
448 whereas the occurrence of petunidin and malvidin xylosides
449 was previously reported in this latter species [25, 33].
450 Aldopentose derivatives were elsewhere detected in
451 *V. uliginosum* berries and identified as xylosides of the five
452 anthocyanidin mentioned above [34]. Accordingly, for

453 delphinidin, petunidin, peonidin, and malvidin aldopentose
454 herein detected, the attribution to xyloside derivatives can be
455 proposed. This putative attribution is also confirmed by the
456 earlier elution of arabinosides compared to the compounds
457 tentatively identified as xylosides [23], being in our study
458 the difference in retention included in the range 3.9–4.4 min.
459 Moreover, considering peaks 30 and 35, retention time was
460 found to be 3.5 and 4.4 min higher than cyanidin arabinoside
461 (peak 18, Table 1), respectively. Accordingly, peak 35
462 (Rt = 21.4 min) should be putatively ascribed to cyanidin-3-
463 *O*-xyloside, whereas peak 30 (Rt = 20.5 min) must be attrib-
464 uted to another cyanidin-aldopentoside, such as cyanidin-7-*O*-
465 arabinoside, which is characterized by a lesser retention under
466 reversed-phase chromatographic conditions and was found in
467 other fruits [29].

468 Peak 38, which was detected only in blueberry, at quite low
469 intensity (about 3000 counts) showed a MS/MS spectra char-
470 acterized by the fragment at *m/z* 331.08, thus indicating a
471 malvidin derivative. The neutral loss from the [M]+ ion was
472 308 Da, which is consistent with a deoxyhexose-hexoside
473 unit, as well as with a coumaroyl-hexoside fragment.
474 However, the [M]+ ion of peak 38 matched the exact mass
475 of a malvidin-deoxyhexose-hexoside with much higher accu-
476 racy ($\Delta = 0.7$ ppm) than a malvidin coumaroyl-hexoside
477 ($\Delta = 34$ ppm). Furthermore, peak 38 eluted between the ara-
478 binoside and xyloside malvidin derivatives, as elsewhere re-
479 ported for malvidin-3-*O*-rutinoside [23], the occurrence of
480 which was previously highlighted in *V. corymbosum* berries,
481 by Ramirez and coworkers [18].

482 Peaks 33, 40, 41, 43, 46, 47, 49, 50, 52, and 53 were
483 characterized by the neutral loss of 204 Da (consistent with
484 an acetyl-hexose unit) and formation of the aglycone frag-
485 ment, thus suggesting their attribution to acetyl-hexosides of

t3.1 **Table 3** Retention times (Rt, min), quasi-molecular ions (TOF MS, Da), main mass fragments (Q/TOF MS/MS, Da), proposed formula, corresponding exact mass (Da), and accuracy ($\Delta$, ppm) of peaks tentatively identified as flavanols in *V. myrtillus* (M), *V. uliginosum* L. subsp. *gaultherioides* (G), and *V. corymbosum* (C) under negative ionization. Mass fragments in italics refer to the most intense signals. Symbols "+" and "−" mean detected and not detected

| | Peak | Rt | TOF MS | Q/TOF MS/MS | Proposed formula | Exact mass | $\Delta$ | M | G | C | Tentative Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t3.3 | 120 | 3.6 | 609.1247[a] | 441.0775; *423.0701*; 305.0639; 177.0200; 125.0250 | $C_{30}H_{26}O_{14}$ | 609.1250 | −0.4 | + | + | − | B-type (E)GC-(E)GC (I) |
| t3.4 | 121 | 4.2 | 305.0661[a] | 219.0667; 167.0345; 165.0182; 139.0391; 137.0245; *125.0239* | $C_{15}H_{14}O_7$ | 305.0667 | −1.7 | + | + | + | Gallocatechin |
| t3.5 | 122 | 4.7 | 609.1237[a] | 441.0814; *423.0709*; 305.0645; 177.0187; 125.0226 | $C_{30}H_{26}O_{14}$ | 609.1250 | −2.2 | + | − | + | B-type (E)GC-(E)GC (II) |
| t3.6 | 123 | 5.8 | 1167.2377[a] | 981.1887; *863.1795*; 711.1422; 573.1045; 411.0698 | $C_{60}H_{48}O_{25}$ | 1167.2412 | −3.0 | + | + | − | A/B-type (E)C-(E)C-(E)C-(E)GC |
| t3.7 | 124 | 6.5 | 1151.2463[a] | *863.1837*; 711.1409; 573.1057; 411.0717 | $C_{60}H_{48}O_{24}$ | 1151.2422 | −3.6 | + | + | − | A/B-type (E)C-(E)C-(E)C-(E)C (I) |
| t3.8 | 125 | 6.7 | 911.1676[a] | *743.1238*; 483.0904; *427.0650*; 423.0672; *305.0637*; 301.0308 | $C_{45}H_{36}O_{21}$ | 911.1646 | −3.3 | + | − | − | A/B-type (E)GC-(E)GC-(E)GC |
| t3.9 | 126 | 6.8 | 451.1229[a] | *289.0719*; 245.0780; 123.0460 | $C_{21}H_{24}O_{11}$ | 451.1246 | −3.7 | + | + | + | Catechin-hexoside |
| t3.10 | 127 | 7.0 | 1153.2619[a] | 1027.2401; 863.1865; 577.1324; *575.1191*; 287.0543 | $C_{60}H_{50}O_{24}$ | 1153.2588 | −2.7 | − | − | + | B-type (E)C-(E)C-(E)C-(E)C (I) |
| t3.11 | 128 | 7.1 | 913.1833[a] | 609.1281; 541.0794; *423.0702*; 305.0641 | $C_{45}H_{38}O_{21}$ | 913.1802 | −3.4 | + | + | − | B-type (E)GC-(E)GC-(E)GC |
| t3.12 | 129 | 7.5 | 881.1900[a] | 713.1523; *695.1375*; 591.1141; 577.1326; 451.1031; 303.0479 | $C_{45}H_{38}O_{19}$ | 881.1935 | −3.9 | − | − | + | B-type (E)C-(E)C-(E)GC (I) |
| t3.13 | 130 | 7.7 | 584.1232[b] | 577.1389; *289.0701*; 287.0542 | $C_{60}H_{50}O_{25}$ | 584.1248 | −2.8 | − | − | + | B-type (E)C-(E)C-(E)C-(E)GC (I) |
| t3.14 | 131 | 7.8 | 727.1469[b] | 591.1163; *289.0704*; 125.0236 | $C_{75}H_{60}O_{31}$ | 727.1486 | −2.5 | + | + | − | A/B-type (E)C-(E)C-(E)C-(E)GC |
| t3.15 | 132 | 8.0 | 895.1704[a] | *727.1298*; 467.0960; *427.0654*; 289.0691; 177.0183 | $C_{45}H_{36}O_{20}$ | 895.1727 | −2.6 | + | − | − | A/B-type (E)C-(E)GC-(E)GC (I) |
| t3.16 | 133 | 8.5 | 577.1335[a] | 425.0869; *407.0766*; 289.0711 | $C_{30}H_{26}O_{12}$ | 577.1351 | −2.9 | + | + | + | Procyanidin B1 [c] |
| t3.17 | 134 | 8.7 | 895.1705[a] | 725.1105; *467.0955*; 427.0676; 305.0661; 125.0238 | $C_{45}H_{36}O_{20}$ | 895.1727 | −2.5 | + | − | − | A/B-type (E)GC-(E)C-(E)GC (II) |
| t3.18 | 135 | 8.9 | 305.0663[a] | 219.0653; 167.0340; 165.0192; 139.0391; 137.0240; *125.0241* | $C_{15}H_{14}O_7$ | 305.0667 | −1.2 | + | + | + | Epigallocatechin |
| t3.19 | 136 | 9.0 | 289.0711[a] | *245.0811*; 205.0501; *203.0712*; 125.0238; *123.0451*; 109.0294 | $C_{15}H_{14}O_6$ | 289.0718 | −2.2 | + | + | + | (+)-Catechin [c] |
| t3.20 | 137 | 9.2 | 719.1489[b] | 575.1182; 451.1036; 411.0698; *289.0701*; *287.0550*; *125.0243* | $C_{75}H_{60}O_{30}$ | 719.1512 | −3.2 | + | + | − | A/B-type (E)C-(E)C-(E)C-(E)C (I) |
| t3.21 | 138 | 9.3 | 1153.2571[a] | 865.2038; *575.1184*; 287.0549 | $C_{60}H_{50}O_{24}$ | 1153.2619 | −4.2 | − | − | + | B-type (E)C-(E)C-(E)C-(E)C (II) |
| t3.22 | 139 | 9.3 | 897.1852[a] | 711.1469; *593.1353*; 543.0920; *407.0776*; 303.0499; 177.0249 | $C_{45}H_{38}O_{20}$ | 897.1884 | −3.5 | + | + | − | B-type (E)GC-(E)C-(E)GC (I) |
| t3.23 | 140 | 9.5 | 865.1957[a] | 695.1393; *577.1343*; 407.0755; *287.0546* | $C_{45}H_{38}O_{18}$ | 865.1985 | −3.3 | + | + | + | B-type (E)C-(E)C-(E)C (I) |
| t3.24 | 141 | 9.8 | 577.1336[a] | 425.0869; *407.0760*; 289.0713 | $C_{30}H_{26}O_{12}$ | 577.1351 | −2.7 | + | + | + | B-type procyanidin (I) |
| t3.25 | 142 | 10.0 | 879.1757[a] | *727.1312*; 451.1026; *427.0655*; 289.0713 | $C_{45}H_{36}O_{19}$ | 879.1778 | −2.4 | + | + | − | A/B-type (E)GC-(E)C-(E)C |
| t3.26 | 143 | 10.2 | 720.1573[b] | 407.0819; *289.0701*; 287.0554 | $C_{75}H_{62}O_{30}$ | 720.1590 | −2.4 | − | − | + | B-type (E)C-(E)C-(E)C-(E)C (I) |
| t3.27 | 144 | 10.3 | 865.1965[a] | *577.1344*; *575.1179*; 287.0544 | $C_{45}H_{38}O_{18}$ | 865.1985 | −2.3 | − | − | + | B-type (E)C-(E)C-(E)C (II) |
| t3.28 | 145 | 10.4 | 451.1237[a] | 289.0707; 245.0802; 125.0247 | $C_{21}H_{24}O_{11}$ | 451.1246 | −2.0 | + | + | + | Epicatechin-hexoside |
| t3.29 | 146 | 10.7 | 720.1579[b] | 577.1352; 407.0765; *289.0701*; 287.0572; 125.0229 | $C_{75}H_{62}O_{30}$ | 720.1590 | −1.5 | − | − | + | B-type (E)C-(E)C-(E)C-(E)C (II) |
| t3.30 | 147 | 10.8 | 576.1258[b] | *449.0874*; 289.0698; 287.0563; *125.0259* | $C_{60}H_{50}O_{24}$ | 576.1273 | −2.6 | − | − | + | B-type (E)C-(E)C-(E)C-(E)C (III) |
| t3.31 | 148 | 10.9 | 865.1962[a] | *577.1353*; *575.1171*; 425.0870; *407.0752*; 287.0542 | $C_{45}H_{38}O_{18}$ | 865.1985 | −2.7 | + | + | + | B-type (E)C-(E)C-(E)C (III) |
| t3.32 | 149 | 11.0 | 897.1864[a] | *711.1323*; 593.1368; 591.1121; 423.0715 | $C_{45}H_{38}O_{20}$ | 897.1884 | −2.2 | + | + | − | B-type (E)GC-(E)C-(E)GC (II) |
| t3.33 | 150 | 11.5 | 720.1568[b] | 575.1249; 405.0628; *289.0702*; 243.0290; 125.0244 | $C_{75}H_{62}O_{30}$ | 720.1590 | −3.1 | − | − | + | B-type (E)C-(E)C-(E)C-(E)C (III) |
| t3.34 | 151 | 11.7 | 576.1260[b] | 425.0828; *289.0603*; 287.0545; 245.0436; *125.0229* | $C_{60}H_{50}O_{24}$ | 576.1273 | −2.3 | + | + | + | B-type (E)C-(E)C-(E)C-(E)C (IV) |
| t3.35 | 152 | 11.8 | 879.1755[a] | *727.1377*; 709.1207; 467.0984; *411.0685*; 305.0644 | $C_{45}H_{36}O_{19}$ | 879.1778 | −2.6 | + | + | − | A/B-type (E)GC-(E)C-(E)GC |
| t3.36 | 153 | 12.2 | 577.1346[a] | 425.0870; *407.0762*; 289.0704 | $C_{30}H_{26}O_{12}$ | 577.1351 | −1.0 | + | + | + | Procyanidin B2 [c] |

t3.37 **Table 3** (continued)

| Peak | Rt | TOF MS | Q/TOF MS/MS | Proposed formula | Exact mass | Δ | M | G | C | Tentative Identification |
|---|---|---|---|---|---|---|---|---|---|---|
| t3.38 154 | 12.6 | 576.1259[b] | 425.0895; 407.0774; *289.0717*; 287.0543; *125.0230* | $C_{60}H_{50}O_{24}$ | 576.1273 | −2.5 | + | + | + | B-type (E)C-(E)C-(E)C-(E)C (V) |
| t3.39 155 | 13.3 | 576.1661[b] | 407.0732; *289.0706*; 287.0542; 151.0376; *125.0241* | $C_{60}H_{50}O_{24}$ | 576.1273 | −2.1 | + | + | + | B-type (E)C-(E)C-(E)C-(E)C (VI) |
| t3.40 156 | 13.9 | 863.1816[a] | 711.1353; 693.1265; 573.1040; 451.1048; *411.0716*; 289.0707 | $C_{45}H_{36}O_{18}$ | 863.1829 | −1.5 | + | + | − | A/B-type (E)C-(E)C-(E)C (I) |
| t3.41 157 | 14.0 | 865.1959[a] | *713.1508*; 695.1377; *577.1352*; 575.1213; 407.0788; 287.0582 | $C_{45}H_{38}O_{18}$ | 865.1985 | −3.1 | − | − | + | B-type (E)C-(E)C-(E)C (IV) |
| t3.42 158 | 14.1 | 720.1570[b] | 289.0710; *125.0235* | $C_{75}H_{62}O_{30}$ | 720.1590 | −3.5 | − | − | + | B-type (E)C-(E)C-(E)C-(E)C-(E)C (IV) |
| t3.43 159 | 14.2 | 289.0718[a] | *245.0818*; 205.0499; *203.0705*; 125.0233; *123.0448*; 109.0299 | $C_{15}H_{14}O_6$ | 289.0718 | 0.1 | + | + | + | (−)-Epicatechin [c] |
| t3.44 160 | 14.3 | 881.1909[a] | 713.1545; *695.1432*; 591.1146; 577.1374; 425.0868; 303.0491 | $C_{45}H_{38}O_{19}$ | 881.1935 | −2.9 | + | + | + | B-type (E)C-(E)C-(E)GC (II) |
| t3.45 161 | 14.5 | 720.1581[b] | 575.1223; *289.0701*; 125.0249 | $C_{75}H_{62}O_{30}$ | 720.1590 | −1.3 | + | + | + | B-type (E)C-(E)C-(E)C-(E)C-(E)C (V) |
| t3.46 162 | 15.4 | 1169.2538[a] | 865.2056; 739.1659; 591.1202; 423.0709; 287.0549 | $C_{60}H_{50}O_{25}$ | 1169.2568 | −2.6 | + | + | + | B-type (E)C-(E)C-(E)C-(E)GC (II) |
| t3.47 163 | 15.5 | 575.1189[b] | 411.0730; 407.0722; *289.0697*; 151.0389; *125.0235* | $C_{60}H_{48}O_{24}$ | 575.1195 | −1.0 | + | + | − | A/B-type (E)C-(E)C-(E)C-(E)C (II) |
| t3.48 164 | 15.5 | 865.1962[a] | 695.1408; *577.1339*; 575.1191; 407.0752; 289.0686; *287.0538* | $C_{45}H_{38}O_{18}$ | 865.1985 | −2.7 | + | + | + | B-type (E)C-(E)C-(E)C (V) |
| t3.49 165 | 16.2 | 576.1255[b] | *407.0756*; *289.0713*; 287.0556; *125.0247* | $C_{60}H_{50}O_{24}$ | 576.1273 | −3.2 | + | + | + | B-type (E)C-(E)C-(E)C-(E)C (VII) |
| t3.50 166 | 16.5 | 720.1578[b] | 289.0707; 125.0238 | $C_{75}H_{62}O_{30}$ | 720.1590 | −1.6 | + | + | + | B-type (E)C-(E)C-(E)C-(E)C-(E)C (VI) |
| t3.51 167 | 16.7 | 720.1583[b] | 289.0712; 125.0244 | $C_{75}H_{62}O_{30}$ | 720.1590 | −0.9 | + | + | + | B-type (E)C-(E)C-(E)C-(E)C-(E)C (VII) |
| t3.52 168 | 17.8 | 863.1826[a] | 711.1386; 693.1277; *575.1197* | $C_{45}H_{36}O_{18}$ | 863.1829 | −0.3 | − | + | − | A/B-type (E)C-(E)C-(E)C (II) |
| t3.53 169 | 18.1 | 719.1500[b] | 411.0728; *289.0684*; 287.0578 | $C_{75}H_{60}O_{30}$ | 719.1512 | −1.6 | + | + | − | A/B-type (E)C-(E)C-(E)C-(E)C-(E)C (II) |
| t3.54 170 | 21.0 | 575.1191[a] | *449.0856*; *423.0718*; *289.0706*; 285.0383 | $C_{30}H_{24}O_{12}$ | 575.1195 | −0.7 | + | + | − | Procyanidin A2 [c] |
| t3.55 171 | 22.0 | 863.1812[a] | 711.1381; 693.1276; *575.1184* | $C_{45}H_{36}O_{18}$ | 863.1829 | −2.0 | − | + | − | A/B-type (E)C-(E)C-(E)C (III) |
| t3.56 172 | 22.7 | 577.1343[a] | 425.0873; *407.0759*; *289.0704* | $C_{30}H_{26}O_{12}$ | 577.1351 | −1.4 | + | + | + | B-type procyanidin (II) |
| t3.57 173 | 27.0 | 576.1267[b] | 407.0749; 287.0517; 151.0388; *125.0233* | $C_{60}H_{50}O_{24}$ | 576.1273 | −1.0 | + | + | + | B-type (E)C-(E)C-(E)C-(E)C (VIII) |

*(E)C* (epi)catechin, *(E)GC* (epi)gallocatechin

[a] Mono-charged quasi-molecular ion [M−H]⁻

[b] Double-charged quasi-molecular ion $[M-2H]^{2-}/2$

[c] Confirmed by spiking the extracts with authentic standards

delphinidin (peaks 33 and 41), cyanidin (peaks 40 and 47), petunidin (peaks 43 and 50), peonidin (peaks 46 and 52), and malvidin (peaks 49 and 53). Interestingly, these acylated anthocyanins showed much higher intensities in blueberry ($1.0 \times 10^4$–$1.4 \times 10^5$ counts) than in bilberry ($4.0 \times 10^3$–$1.0 \times 10^4$ counts) [29], whereas they were never detected in false bilberry (Table 1), thus representing a potential group of markers for the differentiation of these fruit species.

Peaks 39, 42, and 48 were also characteristic of blueberry, which were absent in bilberries and "false bilberries." The MS/MS spectra evidenced the presence of cyanidin and malvidin aglycone fragments as a consequence of the communal loss of 248 Da, which can be ascribed to a malonyl-hexose group. These peaks were therefore tentatively assigned to cyanidin-malonyl-hexoside (peak 39) and malvidin-malonyl-hexosides (peaks 42 and 48), in partial agreement with the results obtained by Wu and Prior [29], which reported the occurrence of malonyl-glucoside derivatives of delphinidin, cyanidin, and malvidin in blueberry.

Peaks 51, 54–60, 62, and 63 fragmented with a neutral loss of 308 Da and the resulting formation of ions at m/z 303.05 (i.e., delphinidin, peaks 51 and 55), m/z 287.05 (i.e., cyanidin, peaks 54 and 57), m/z 317.07 (i.e., petunidin, peaks 56 and 59), m/z 301.07 (i.e., peonidin,

t4.1 **Table 4** Retention times (Rt, min), [M−H]⁻ quasi-molecular ions (TOF MS, Da), main mass fragments (Q/TOF MS/MS, Da), proposed formula, corresponding exact mass (Da), and accuracy (Δ, ppm) of peaks tentatively identified as other phenolic compounds in *V. myrtillus* (M), *V. uliginosum* L. subsp. *gaultherioides* (G), and *V. corymbosum* (C) under negative ionization. Mass fragments in italics refer to the most intense signals. Symbols "+" and "−" mean detected and not detected

| Peak | Rt | TOF MS | Q/TOF MS/MS | Proposed formula | Exact mass | Δ | M | G | C | Tentative identification |
|---|---|---|---|---|---|---|---|---|---|---|
| 174 | 2.0 | 169.0146 | *125.0240*; 124.0244; 79.0189 | $C_7H_6O_5$ | 169.0142 | 2.0 | + | + | + | Gallic acid[a] |
| 175 | 6.8 | 353.0866 | *191.0554*; 179.0345; 135.0446 | $C_{16}H_{18}O_9$ | 353.0878 | −3.4 | + | + | + | Neochlorogenic acid[a] |
| 176 | 9.2 | 337.0918 | 191.0562; *163.0390*; 119.0499 | $C_{16}H_{18}O_8$ | 337.0929 | −3.2 | − | − | + | Coumaroylquinic acid (I) |
| 177 | 9.4 | 337.0916 | *191.0554*; 163.0387; 119.0505 | $C_{16}H_{18}O_8$ | 337.0929 | −3.7 | − | − | + | Coumaroylquinic acid (II) |
| 178 | 10.0 | 179.0351 | *135.0444*; 134.0368 | $C_9H_8O_4$ | 179.0350 | 0.6 | + | + | + | Caffeic acid[a] |
| 179 | 10.6 | 341.0872 | *179.0342*; 135.0446; 134.0358 | $C_{15}H_{18}O_9$ | 341.0878 | −1.7 | + | + | + | Caffeic acid hexoside |
| 180 | 11.0 | 353.0869 | *191.0566* | $C_{16}H_{18}O_9$ | 353.0878 | −2.6 | + | + | + | Chlorogenic acid[a] |
| 181 | 12.3 | 325.0920 | *163.0395*; 119.0504 | $C_{15}H_{18}O_8$ | 325.0929 | −2.6 | + | + | + | Coumaric acid hexoside |
| 182 | 12.4 | 353.0868 | *191.0552*; 179.0348; *173.0451*; 135.0448 | $C_{16}H_{18}O_9$ | 353.0878 | −2.8 | + | + | + | Cryptochlorogenic acid[a] |
| 183 | 13.7 | 355.1031 | 193.0507; *175.0398*; *160.0163*; 132.0210 | $C_{16}H_{20}O_9$ | 355.1035 | −1.0 | + | + | + | Ferulic acid hexoside |
| 184 | 13.9 | 739.1657 | *587.1205*; 339.0494; 289.0707; 177.0190 | $C_{39}H_{32}O_{15}$ | 739.1668 | −1.5 | − | − | + | Cinchonain IIx (I) |
| 185 | 14.7 | 337.0918 | *191.0547*; 163.0384 | $C_{16}H_{18}O_8$ | 337.0929 | −3.3 | + | − | + | Coumaroylquinic acid (III) |
| 186 | 15.4 | 353.0873 | *191.0568* | $C_{16}H_{18}O_9$ | 353.0878 | −1.4 | + | − | + | Caffeoylquinic acid |
| 187 | 16.0 | 739.1658 | 587.1192; 449.0864; 339.0498; *289.0704* | $C_{39}H_{32}O_{15}$ | 739.1668 | −1.5 | − | − | + | Cinchonain IIx (II) |
| 188 | 16.2 | 335.0767 | *179.0343*; 161.0207; *135.0446*; 134.0372 | $C_{16}H_{16}O_8$ | 335.0772 | −1.6 | + | + | + | Caffeic acid derivative |
| 189 | 16.9 | 739.1641 | *587.1170*; 339.0510; *289.0704* | $C_{39}H_{32}O_{15}$ | 739.1668 | −3.8 | + | − | + | Cinchonain IIx (III) |
| 190 | 17.4 | 191.0349 | *176.0109*; 104.0277 | $C_{10}H_8O_4$ | 191.0350 | −0.5 | − | + | − | Scopoletin[a] |
| 191 | 17.5 | 739.1667 | *587.1195*; 449.0871; 339.0484; *289.0688*; *177.0174* | $C_{39}H_{32}O_{15}$ | 739.1668 | −0.2 | + | − | + | Cinchonain IIx (IV) |
| 192 | 17.6 | 367.1025 | 193.0494; *191.0555*; 173.0452; 134.0373 | $C_{17}H_{20}O_9$ | 367.1035 | −2.6 | + | + | + | Feruloylquinic acid |
| 193 | 17.9 | 193.0504 | *134.0367*; 133.0302 | $C_{10}H_{10}O_4$ | 193.0506 | −1.3 | − | − | + | Ferulic acid[a] |
| 194 | 21.2 | 367.1026 | *179.0342*; 161.0256 *135.0446*; 134.0357 | $C_{17}H_{20}O_9$ | 367.1035 | 0.8 | + | + | + | Caffeic acid derivative |
| 195 | 24.4 | 535.1454 | 371.0980; 329.1038; 191.0346; *163.0398*; *147.0450*; 119.0501 | $C_{25}H_{28}O_{13}$ | 535.1457 | −0.5 | + | + | − | Coumaroyl iridoid (I) |
| 196 | 25.7 | 535.1453 | 371.0987; 329.1025; 191.0337; *163.0396*; *147.0443*; 119.0500 | $C_{25}H_{28}O_{13}$ | 535.1457 | −0.7 | + | + | − | Coumaroyl iridoid (II) |
| 197 | 26.2 | 515.1184 | *353.0867*; *191.0555*; *179.0345*; 173.0452; 135.0450 | $C_{25}H_{24}O_{12}$ | 515.1195 | −2.2 | − | − | + | Dicaffeoylquinic acid |
| 198 | 26.8 | 515.1179 | *353.0872*; *191.0550*; 179.0347; 135.0445 | $C_{25}H_{24}O_{12}$ | 515.1195 | −3.0 | − | − | + | 1,5-Dicaffeoylquinic acid[a] |
| 199 | 28.0 | 435.1291 | *273.0751*; 167.0322 | $C_{21}H_{24}O_{10}$ | 435.1297 | −1.3 | + | + | + | Phloridzin[a] |
| 200 | 32.1 | 411.1659 | 163.0402; *145.0290*; 119.0481 | $C_{20}H_{28}O_9$ | 411.1661 | 1.5 | + | + | − | Coumaric acid-malonyl-hexoside (I) |
| 201 | 32.5 | 411.1659 | *163.0398*; *145.0292*; 119.0498 | $C_{20}H_{28}O_9$ | 411.1661 | −0.3 | + | + | − | Coumaric acid-malonyl-hexoside (II) |
| 202 | 32.6 | 445.1143 | *179.0346*; *135.0441*; 134.0367 | $C_{22}H_{22}O_{10}$ | 445.1040 | 0.6 | + | + | + | Caffeic acid derivative |

[a] Confirmed by spiking the extracts with authentic standards

peaks 58 and 62), and *m/z* 331.08 (i.e., malvidin, peaks 60 and 63). The signal intensities of these compounds were much higher in *V. myrtillus* berries (approximately from $1 \times 10^4$ to $1 \times 10^5$ counts) in respect to *V. uliginosum* L. subsp. *gaultherioides* and *V. corymbosum* ones. Based on the [M]⁺ TOF accurate mass values determined for these analytes, the putative attribution to coumaroyl-hexoside anthocyanidin derivatives can be proposed ($\Delta \leq 1.9$ ppm), in agreement with the findings obtained under very similar chromatographic conditions by Zoratti and colleagues in *V. myrtillus* fruits collected in the Alps of Northern Italy [35].

Finally, peaks 61 ($8.6 \times 10^3$ counts) and 64 ($1.7 \times 10^5$ counts), which were peculiar of *V. myrtillus* fruits, exhibited a single neutral loss of 338 Da (i.e., feruloyl-hexoside) with formation of the fragment at *m/z* 331.08 (i.e., malvidin). Accordingly, these peaks were tentatively identified as malvidin-feruloyl-hexosides. It is remarkable that these compounds, previously found in others fruits (e.g., grape) [36], were herein identified in bilberry for the first time.

*Flavonols*

According to literature, the detection of flavonols by ESI-MS techniques can be achieved with high sensitivity under negative ionization mode [37]. Hence, in this study, flavonols were identified via negative polarity, by monitoring the quasi-molecular $[M-H]^-$ ion and its fragments (Table 2). Furthermore, peak assignment was confirmed under positive ionization, by monitoring the quasi-molecular $[M+H]^+$ ion, which allowed to pinpoint all the analytes found by negative mode, even though with lower signal intensity.

Using the IDA TOF-Q/TOF workflow and comparisons among retention times and mass spectra of unknown and authentic standards, the unequivocal or at least tentative identification of 55 flavonols was achieved. As illustrated in Table 2, among the identified flavonols, we found 36 glycosides (peaks 66–71, 74–79, 82, 86–88, 91–94, 96–97, 99–101, 103–104, 107–110, 112–114, 116, and 117), 6 glucuronides (peaks 65, 73, 83, 90, 106, and 111), 9 acyl derivatives (peaks 72, 80, 84, 85, 89, 95, 98, 102, and 105), and 4 aglycones (peaks 81, 115, 118, and 119). TOF MS $[M-H]^-$ quasi-molecular ions matched the proposed formulae with very high mass accuracy, being $\Delta$ absolute values $\leq 2.5$ ppm for 75% of the identified analytes, and included in the range of 2.6–4.4 ppm in the remaining cases (Table 2).

In agreement with literature findings [38], Q/TOF MS/MS spectrum of flavonol glycosides exhibited both the heterolytic and the homolytic cleavage of the glycosidic bond, producing the aglycone fragment ion $[Y_0]^-$ and the radical aglycone ion $[Y_0-H]^-$ $^\bullet$. Figure S5A of the ESM illustrates as an example the MS/MS spectrum of quercetin-3-O-glucoside (peak 75), in which the ions derived from heterolytic ($m/z = 301.03$) and homolytic ($m/z = 300.03$) fission of the glycosidic bond are shown. Neutral losses of 18 Da ($H_2O$), 28 Da (CO), and 30 Da ($CH_2O$), individual or combined one with the other, have been also observed starting from the $[Y_0]^-$ ion (ESM Fig. S5A), in agreement with characteristic MS/MS behavior of flavonols elsewhere reported [37, 39]. Moreover, the loss of 15 Da ($-CH_3$) from the aglycone was occasionally observed and putatively attributed to methoxylated flavonols (e.g., peak 78, tentatively identified as laricitrin-3-O-galactoside, see Fig. S5B of the ESM). Further typical ions, at $m/z = 151.00$ and $m/z = 179.00$, originating from different retrocyclization cleavages of the "C" ring and commonly identified as $^{1,3}A^-$ (retro-Diels-Alder) and $^{1,2}A^-$ fragments [40, 41], were observed, even though with low signal intensity (ESM Fig. S5A, B).

A different relative abundance of the aglycone fragment $[Y_0]^-$ and the aglycone radical $[Y_0-H]^-$ $^\bullet$ ions, resulting from heterolytic and homolytic cleavage of the glycosidic bond, has been elsewhere demonstrated for kaempferol glycosides and suggested also for other flavonol glycosides, on the basis of the linkage position, as well as of the length of the saccharide chain [38]. More in detail, if the sugar is a monosaccharide, the cleavage of the 3-O position of the aglycone gives rise preferentially to the $[Y_0-H]^-$ $^\bullet$ than the $[Y_0]^-$, as observed for example in peaks 66–71 (Table 2). Conversely, in this study, the preferential heterolytic cleavage of the monosaccharide glycosidic bond was never observed, thus excluding the presence of 7-O-glycoside derivatives of flavonols in the investigated *Vaccinium* species. Based on the aforementioned considerations, peaks 66, 67, 78, 82, 86, 93, 97, 103, 107, and 109 can be ascribed to 3-O-monohexoside flavonol derivatives. More in detail, considering the whole mass dataset, as well as the relative peak elution order, 3-O-galactoside and 3-O-glucoside derivatives of myricetin (peaks 66 and 67), laricitrin (peaks 78 and 82), kaempferol (peaks 86 and 93), isorhamnetin (peaks 97 and 103), and syringetin (peaks 107 and 109) can be putatively identified, whereas peaks 71 and 75 were unequivocally identified as quercetin-3-O-galactoside and quercetin-3-O-glucoside, due to the availability of the authentic reference standards (Table 2). These flavonols were found to be present in all the investigated *Vaccinium* species, with the only exception of the glucoside derivative of kaempferol in bilberry and blueberry, and of the glucoside derivative of isorhamnetin in blueberry. It should also be underlined that flavonol glucosides and especially galactosides occurred with much higher signal intensity in *V. uliginosum* L. subsp. *gaultherioides* berries, suggesting that these analytes could be a typical metabolomic trait of "false bilberry."

Peaks 68–70, 77, 79, 87, 88, 91, 94, 99, 110, 112–114, 116, and 117 showed the neutral loss of 132 Da, indicating the presence of the aldopentoside residue, and were attributed to 3-O-aldopentoside derivatives of the aforementioned aglycones, on the basis of exact mass data of pseudo-molecular ions and aglycone fragments. Interestingly, for the most abundant aldopentoside derivatives, a net predominance was observed in "false bilberry," whereas the others generally had higher signal intensities in blueberry.

Peaks 92 and 104 were characterized by the neutral loss of 146 Da (i.e., deoxyhexose unit). The identity of the former peak, which was found in both bilberry and blueberry, was unequivocally attributed to quercetin-3-O-rhamnoside, using the reference standard. Peak 104, which was found only in blueberry, was identified as a laricitrin-deoxyhexoside and putatively attributed to larictrin-3-O-rhamnoside.

Data herein obtained for quercetin-3-O-rutinoside (peak 76, Fig. S6A of the ESM) and kaempferol-7-O-neohesperidoside (peak 96, Fig. S6B of the ESM), which were available as reference standards, suggested, also for flavonol disaccharides, the higher abundance of homolytic or heterolytic cleavages, as diagnostic of the 3-O- or 7-O-substitution, respectively. However, according to Lu and coworkers [38], a long saccharide chain substituted at the 3-O position, could hinder the occurrence of the $[Y_0-H]^-$ $^\bullet$ ion, resulting in

product ion MS/MS spectra similar to those of flavonol-7-*O*-glycosides. Accordingly, even though the differentiation of 3-*O* and 7-*O*-disaccharides of flavonols is commonly performed on this basis [42, 43], their attribution was considered herein as putative. Following this approach, some 3-*O*-disaccharide (peaks 74, 76, 100, and 101) and 7-*O*-disaccharide (peaks 96 and 108) derivatives of various flavonols were detected (Table 2).

The tentative identification of glucuronide derivatives of myricetin (peak 65), quercetin (peak 73, see Fig. S7 of the ESM), laricitrin (peak 83), kaempferol (peak 90), isorhamnetin (peak 106), and syringetin (peak 111) was associated to the neutral loss of 176 Da (i.e., glucopyranuronic acid) and formation of the $[Y_0]^-$ ion, consequent to the heterolytic cleavage of the glucuronic bond, whereas the homolytic fragmentation was absent. This mass behavior was probably due to the lower electrophilic nature of glucuronic acid compared to glucose. Interestingly, the abovementioned glucuronides showed comparable signal intensities in bilberry and "false bilberry," whereas a much lower occurrence was highlighted in blueberry.

Peaks 72, 80, 89, 95, 98, 102, and 105, which were found exclusively in blueberry, were putatively identified as acetyl-hexosides of myricetin, quercetin, and laricitrin. Peaks 84 and 85 were also exclusively present in blueberry and tentatively ascribed to malonyl-hexosides of quercetin (Table 2). These attributions were proposed on the basis of neutral losses of 205/204 Da (homolytic/heterolytic cleavage of the acetyl-hexose unit) or 249/248 Da (homolytic/heterolytic cleavage of the malonyl-hexose group), respectively (Table 2).

Four aglycones were also detected in all the investigated *Vaccinium* species (peaks 81, 115, 118, and 119). These molecules fragmented according to retrocyclization ($^{1,2}A^-$ and $^{1,2}B^-$) and retro-Diels-Alder cleavages ($^{1,3}A^-$ and $^{1,3}B^-$) of the "C" ring and were identified as myricetin, quercetin, laricitrin, and isorhamnetin. Different signal intensities were observed for the four aglycones, with myricetin being the predominant aglycone in bilberry and quercetin the compound more abundant in "false bilberry" and blueberry.

*Flavanols*

Flavanol ESI-MS detection can be achieved both via positive and negative ionization [44]. Accordingly, in this study, the two ionization modes were evaluated for flavanol identification. The results highlighted a slightly better sensitivity using the negative polarity, notwithstanding the high percentage of formic acid used in the eluents that lowered the ionization efficiency under negative potential. The IDA TOF-Q/TOF workflow applied to berry samples and also to some authentic standards allowed for certainly or putatively identifying 54 flavanols with a very good agreement between TOF MS quasi-molecular ions and proposed formulae ($\Delta$ absolute

values ≤4.2 ppm). The identification data obtained with the negative ionization are reported in Table 3.

Peaks 121 and 135 were respectively assigned to gallocatechin (GC) and epigallocatechin (EGC), which are stereoisomers not distinguishable by mass spectrometry, but well-discriminated by reversed-phase LC. These peaks showed $[M-H]^-$ quasi-molecular ion at *m/z* 305.07 and the same MS/MS spectra (see Fig. S8A of the ESM) with main fragments at *m/z* 261.04 (loss of 44 Da, $CH_2$=CHOH), *m/z* 221.05 (cleavage of the "A" ring), *m/z* 219.03 (consecutive losses of 44 and 42 Da), and *m/z* 125, the last being by far the most intense ion of the MS/MS spectrum. The high intensity of this ion can be explained on the basis of its dual origin that is from the fission of the heterocyclic ring or the cleavage of the "B" ring, both characterized by the loss of 180 Da (see fragmentation paths 4 and 8 of Scheme S1 of the ESM). Moreover, in accordance to the findings previously reported for catechin (C) and (EC) [45, 46], fragments at *m/z* 139.04 (probably attributable to the cleavage of the "A" and "C" rings) and *m/z* 137.03 (loss of 168 Da, retro-Diels-Alder reaction) were observed. GC and EGC, herein found in all the investigated *Vaccinium* species, were previously reported only in *V. myrtillus* fruits [44]. When MS/MS spectra of peaks 121 and 135 (ESM Fig. S8A) were compared to the ones of peaks 136 and 159 (ESM Fig. S8B), delta mass of 16 Da was observed in most cases. Spiking procedure of the authentic standards unequivocally confirmed the identification of the latter peaks as C and EC (see also ESM Scheme S1 for detailed fragmentation paths). The predominance of catechin in *V. corymbosum* fruits and of epicatechin in *V. myrtillus* and *V. uliginosum* L. subsp. *gaultherioides* berries has been observed, in agreement with the findings already reported in the literature [4, 7].

MS/MS spectra of peaks 126 and 145 revealed the loss of 162 Da (hexose unit) with formation of the ion at *m/z* 289.07, which is attributable to both C or EC, due to their stereoisomeric nature (following the possible presence of the C or the EC unit is indicated as (E)C). Moreover, the aforementioned typical fragments of (E)C were observed, thus indicating the presence of (E)C-hexosides, never reported in *Vaccinium* species, but previously identified in other berries [15].

Peaks 133 and 153 exhibited the typical fragmentation of B-type (E)C-(E)C dimers, consisting in the retro-Diels-Alder fission of the "C" ring (*m/z* 425.09) and successive loss of water (*m/z* 407.08), as well as the cleavage of the B-type linkage with formation of the (E)C monomer (*m/z* 289.07). These peaks were undoubtedly attributed to procyanidin B1 and procyanidin B2, respectively, on the basis of identity confirmation with authentic standards. Peaks 141 and 172 showed the same MS/MS spectrum and were therefore putatively ascribed to B-type procyanidin isomers, in which the C4→C6 interflavanoid bond, instead of the C4→C8 one, is present between the two (E)C units.

The comparison between MS/MS spectra of B-type procyanidins and peaks 120 and 122 highlighted $m/z$ values 16 Da higher in most detected fragments of the latter peaks (i.e., $m/z$ 441.08, 423.07, and 305.06, see Fig. 1). Accordingly, peaks 120 and 122 were tentatively identified as B-type (E)GC-(E)GC dimers (Table 3). It is remarkable that for these peaks, the fragment at $m/z$ 177.02 has been also observed, in contrast to MS/MS findings of (E)C dimers, in which this ion was absent. It should also be noted that the fragment at $m/z$ 177.02 was absent in (E)GC (see ESM Fig. S8A), thus suggesting that it derives from the $m/z$ 303.05 ion, as proposed in Scheme 1.

Peak 170 showed the typical fragmentation of A-type procyanidins (e.g., cleavage of the "C" ring, fission of the heterogeneous C2→O interflavanyl linkage and rearrangement with formation of the ion at $m/z$ 449.09), which was unequivocally identified as procyanidin A2, based on its authentic standard.

A number of proanthocyanidin trimers, tetramers, and pentamers, characterized by B-type and both A- and B-type (following A/B) interflavanyl linkages, were identified (Table 3) on the basis of the typical mass fragmentation mechanisms of this polyphenol class: retro-Diels-Alder (RDA), quinone methide formation (QM), and heterocyclic ring fissions (HRF) [47].

Most of these proanthocyanidins were trimers (peaks 140, 144, 148, 157, and 164), tetramers (peaks 127, 138, 147, 151, 154, 155, 165, and 173), and pentamers (peaks 143, 146, 150, 158, 161, 166, and 167) consisting of only B-linked (E)C units. More in detail, the MS/MS spectrum of B-type procyanidin trimers was characterized by ions derived from RDA fission ($m/z$ 713.15) and successive loss of water ($m/z$ 695.14), as well as the typical fragmentation pattern of B-type procyanidin dimers (i.e., $m/z$ 425.09 and 407.08) previously discussed. Moreover, the presence of monomer ($m/z$ 289.07 and 287.06) and dimer (E)C units ($m/z$ 577.13 and 575.12) derived from QM reaction confirmed the identity of B-type procyanidin trimers. B-type procyanidin tetramers were detected both as mono-charged ($m/z$ 1153.26) and double-charged ($m/z$ 576.13) quasi-molecular ions and produced fragments related to trimeric ($m/z$ = 865.20 and 863.19), dimeric ($m/z$ = 577.13 and 575.12), and monomeric ($m/z$ = 289.07 and 287.07) units, up to the characteristic MS/MS spectra of (E)C. Similarly, B-type procyanidin pentamers showed the typical fragmentations of the lower molecular weight B-type procyanidin oligomers.

A/B-linked proanthocyanidin trimers (peaks 156, 168, and 171), tetramers (peaks 124 and 163), and pentamers (peaks 137 and 169) formed only of (E)C units were also putatively identified. For these compounds, the RDA reaction affecting the B-type-linked (E)C caused the ion at $m/z$ 711.14 and, after the loss of water, the ion at $m/z$ = 693.13. The QM reaction produced the fragments at $m/z$ 573.10 and 289.07, whereas the ions at 451.10 and 411.07 derived from HRF reactions. Similarly, A/B-type procyanidin tetramers and pentamers were identified on the basis of their MS/MS fragments consisting of the abovementioned typical product ions of A/B-type procyanidin trimer ($m/z$ = 863.18) and dimer ($m/z$ = 575.12).

Interestingly, two compounds exclusively formed by (E)GC units (i.e., peak 125, A/B-type trimer, and peak 128, B-type trimer) were herein identified for the first time in *V. myrtillus* and *V. uliginosum L.* subsp. *gaultherioides* fruits. Peak 125 was identified as A/B-type prodelphinidin trimer on the basis of the characteristic fragments derived from RDA ($m/z$ = 743.13) and HRF ($m/z$ 483.09 and 427.07) reactions, whereas the MS/MS spectrum of peak 128 exhibited the product ions corresponding to the formation of dimer ($m/z$ 609.13) and monomer ($m/z$ 305.07) ions. In addition, the ion at $m/z$ 541.08, derived from the cleavage of the "B" rings of the trimer, was observed.

Four B-type proanthocyanidin trimers (peaks 129, 139, 149, and 160) and two tetramers (peaks 130 and 162) formed by both (E)C and (E)GC monomers were also identified (Table 3), but no information about the relative position of the different units could be obtained by the MS/MS spectra.

Finally, six proanthocyanidin oligomers were identified as trimers, tetramers, and pentamers consisting of both (E)C and (E)GC units, linked with A/B-type (peaks 123, 131, 132, 134, 142, and 152) bonds. Interestingly, in this case, the fragmentation spectra highlighted in most cases the diagnostic ions that indicated the relative position of a certain monomer and/or the type of linkage (A type or B type) between two monomers. For instance, peaks 142 and 152 were identified as A/B-type trimers constituted by two units of (E)C and one unit of (E)GC. For both peaks, the RDA reaction and the successive loss of water, producing the ions at $m/z$ 727.13 and 709.12, were observed. Nevertheless, peak 142 was characterized by fragments at $m/z$ 427.07 and 451.10, fully consistent with the occurrence in the molecule of (i) one terminal (E)GC linked to one (E)C by an A-type linkage and (ii) two (E)C units linked by a B-type interflavanyl bond, respectively. Moreover, a high-intensity fragment at $m/z$ 289.07 was observed, in accordance with the presence of a B-type terminal (E)C (Fig. 2A). Analogously, the MS/MS spectrum of peak 152 showed fragments at $m/z$ 411.07 and 467.10, which are in agreement with the presence of (i) one terminal (E)C linked to the other portion of the molecule by an A-type linkage and (ii) one (E)C and one (E)GC unit linked each other by a B-type bond. Moreover, it should be noted that an intense fragment at $m/z$ 305.06, attributable to the (E)GC unit, was also observed (Fig. 2B). Accordingly, peaks 142 and 152 were putatively identified as A/B-type (E)GC-(E)C-(E)C and A/B-type (E)C-(E)C-(E)GC, respectively.

A similar consideration can be done for peaks 132 and 134, which were A/B-type trimer constituted by one unit of (E)C and

**Fig. 1** Q/TOF MS/MS spectrum of peaks 120 and 122, identified as B-type dimer of (epi)gallocatechin
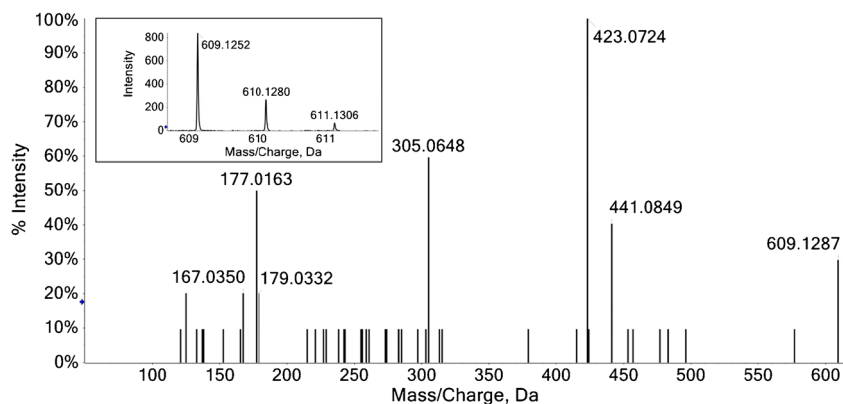
845 two units of (E)GC. In both these peaks, the ion at $m/z$ 427.07
846 indicated the presence of one terminal (E)GC unit linked with an
847 A-type bond with the rest of the molecule, whereas the ion at $m/z$
848 467.10 was diagnostic for the occurrence of B-type-linked
849 (E)GC and (E)C. However, peak 132 was also characterized by
850 the presence of an intense fragment at $m/z$ 289.07, differently
851 from peak 142 that showed a high-intensity ion at $m/z$ 305.07.
852 Therefore, peaks 132 and 134 were putatively identified as A/B-
853 type (E)GC-(E)GC-(E)C and A/B-type (E)GC-(E)C-(E)GC, re-
854 spectively (see Fig. 3A, B).
855   Peak 123 was identified as an A/B-type tetramer formed by
856 three units of (E)C and one unit of (E)GC. This peak

857 fragmented originating the ion at $m/z$ 863.18, which is consis-
858 tent with the formation of an (epi)catechin trimer with one A-
859 type and one B-type linkage, together with other characteristic
860 ions (i.e., $m/z$ 711.14, 573.10, and 411.07), deriving from the
861 catechin trimer fragmentation. Accordingly, in peak 123, the
862 (E)GC unit should be terminal and linked through a B-type
863 interflavanyl linkage.
864   Finally, peak 131, which was formed by four units of (E)C
865 and one unit of (E)GC, two of them linked by an A-type
866 interflavanyl bond, showed, among other, a quite intense frag-
867 ment at $m/z$ 591.12. This ion is compatible with the presence
868 of an A-type bond between the (E)GC and one (E)C units.
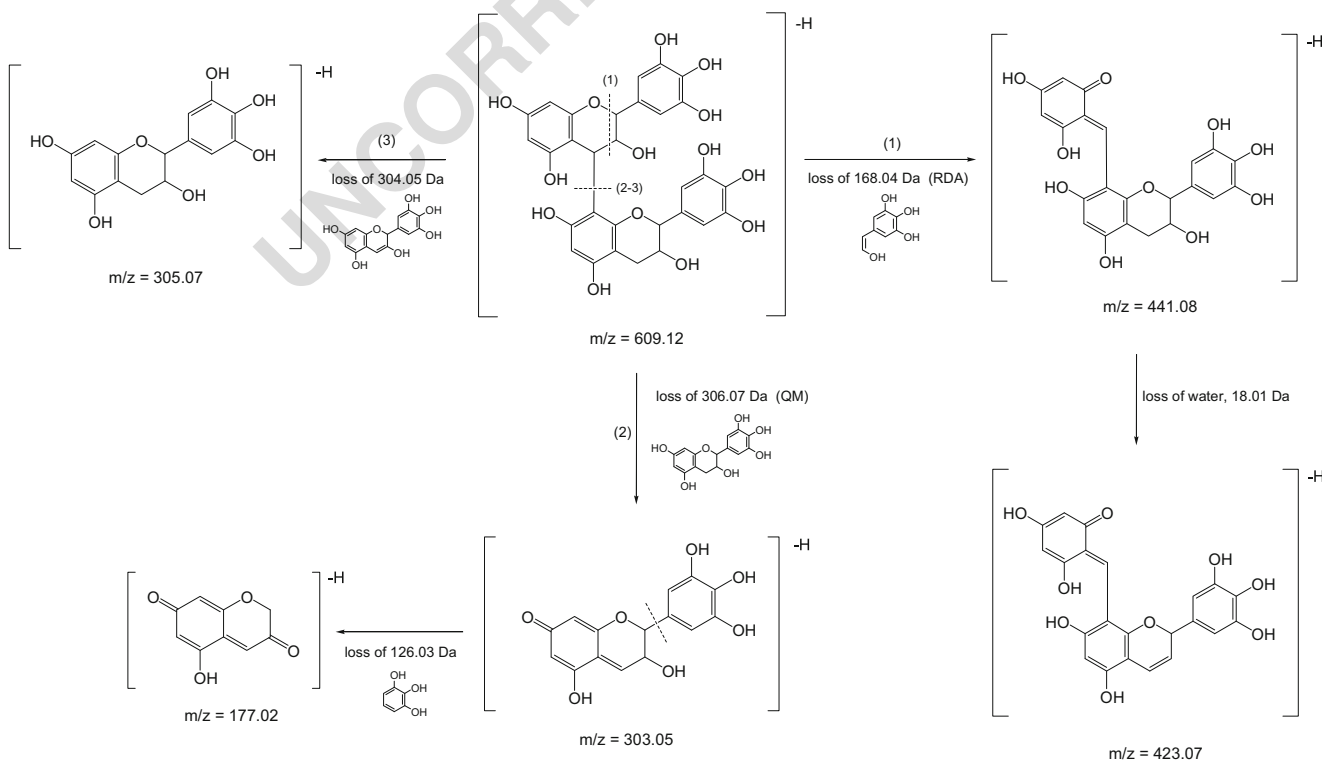


**Scheme 1** Hypothesized structure and fragmentation scheme for peaks 120 and 122 ([M−H]⁻ = 609.12, putatively attributed to an (epi)gallocatechin dimer. *RDA* retro-Diels-Alder, *QM* quinone methide formation

**Fig. 2** Q/TOF MS/MS spectrum of peaks (**A**) 142 and (**B**) 152, identified as A/B-type (E)GC-(E)C-(E)C and A/B-type (E)C-(E)C-(E)GC, respectively. *(E)C* (epi)catechin, *(E)GC* (epi)gallocatechin. Note that ion $^{1,3}B^-$ is not evidenced in the mass spectra
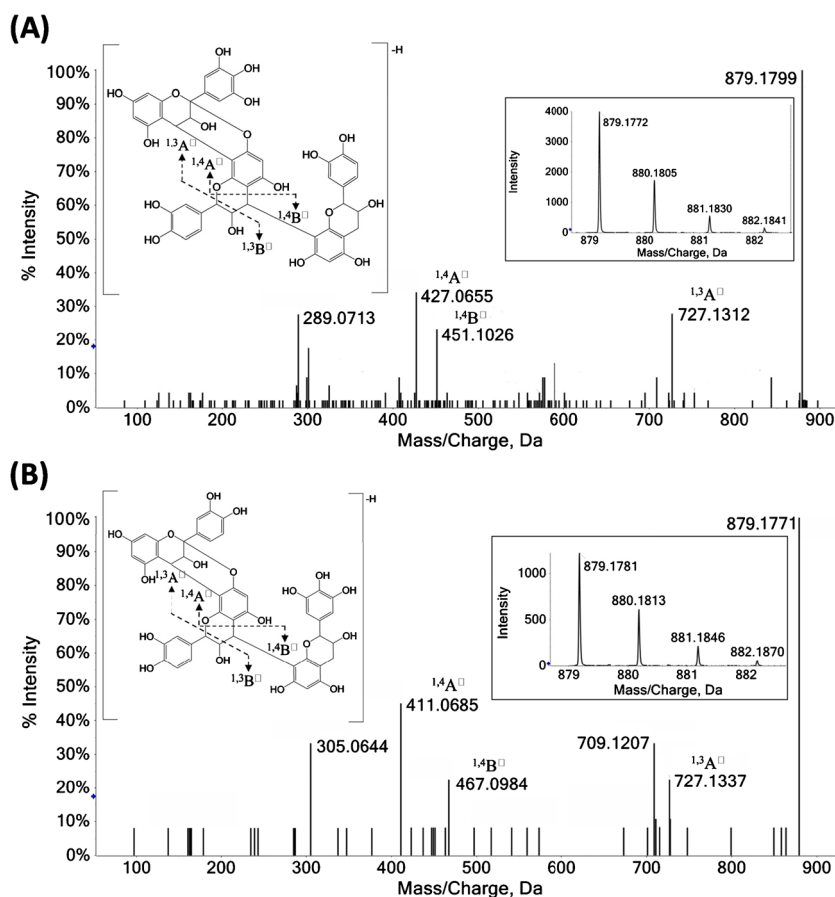
### Other compounds

Using the IDA TOF-Q/TOF workflow under negative ionization, 29 further phenolic compounds belonging to various classes were putatively or unequivocally identified in berry samples (Table 4). Also in these cases, a very good agreement between TOF MS quasi-molecular ions and proposed formulae was obtained ($\Delta$ absolute values ≤3.8 ppm).

Peak 199 was found to be common to the three species and unequivocally identified as phloridzin after comparison with the corresponding authentic standard. The use of the reference standard allowed for certainly identifying also peak 190 as scopoletin, which was detected only in "false bilberry."

Peak 174, which was present at quite similar intensities in all berry species, was unambiguously identified as gallic acid, due to the availability of the authentic reference standard.

Several phenolic acids belonging to the class of the hydroxycinnamic acids (peaks 175–178, 180, 182, 185, 186, 192, 193, 197, and 198) were also putatively or unequivocally identified, depending on the availability of the authentic standards. These compounds were generally found at higher intensity in *V. corymbosum* and in some cases (peaks 176, 177, 193, 197, and 198) detected exclusively in this berry species.

Peak 179 exhibited a quasi-molecular ion at *m/z* 353.08, which fragmented giving rise to a neutral loss of 162 Da and

ions attributable to caffeic acid. This peak was therefore putatively attributed to a caffeic acid hexoside. Analogously, peaks 181 and 183 were tentatively identified as coumaric acid and ferulic acid hexosides.

Peaks 188, 194, and 202 showed pseudo-molecular ions at *m/z* 335.08, 367.10, and 445.11, respectively, and shared the typical fragments of caffeic acid (Table 4), thus suggesting their putative attribution as caffeic acid derivatives.

For peaks 201 and 202, the same quasi-molecular ion at *m/z* 411.17 was found. The fragmentation gave rise to a neutral loss that corresponded to a malonyl hexoside (248 Da) with formation of a fragment consistent with coumaric acid [M−H]⁻ ion (*m/z* 163.04), as well as various fragments typical of coumaric acid, thus suggesting the putative identification of both peaks as coumaric acid-malonyl-hexosides.

Fragmentations of the quasi-molecular [M−H]⁻ ions of peaks 195 and 196 (*m/z* 535.15) were in full agreement with data reported by Hokkanen et al. [44] for coumaroyl iridoids. These peaks, previously identified in *V. myrtillus* fruits [48], were much more intense in bilberry than in "false bilberry," whereas these were absent in blueberry.

Peaks 184, 187, 189, and 191 were putatively identified as cinchonain II isomers, in agreement with the fragmentation scheme reported by Hokkanen and coworkers [44]; all these compounds have been found in *V. corymbosum* berries,

**Fig. 3** Q/TOF MS/MS spectrum of peaks (**A**) 132 and (**B**) 134, identified as A/B-type (E)GC-(E)GC-(E)C and A/B-type (E)GC-(E)C-(E)GC, respectively. *(E)C* (epi)catechin, *(E)GC* (epi)gallocatechin. Note that ion $^{1,3}B^-$ is not evidenced in the mass spectra
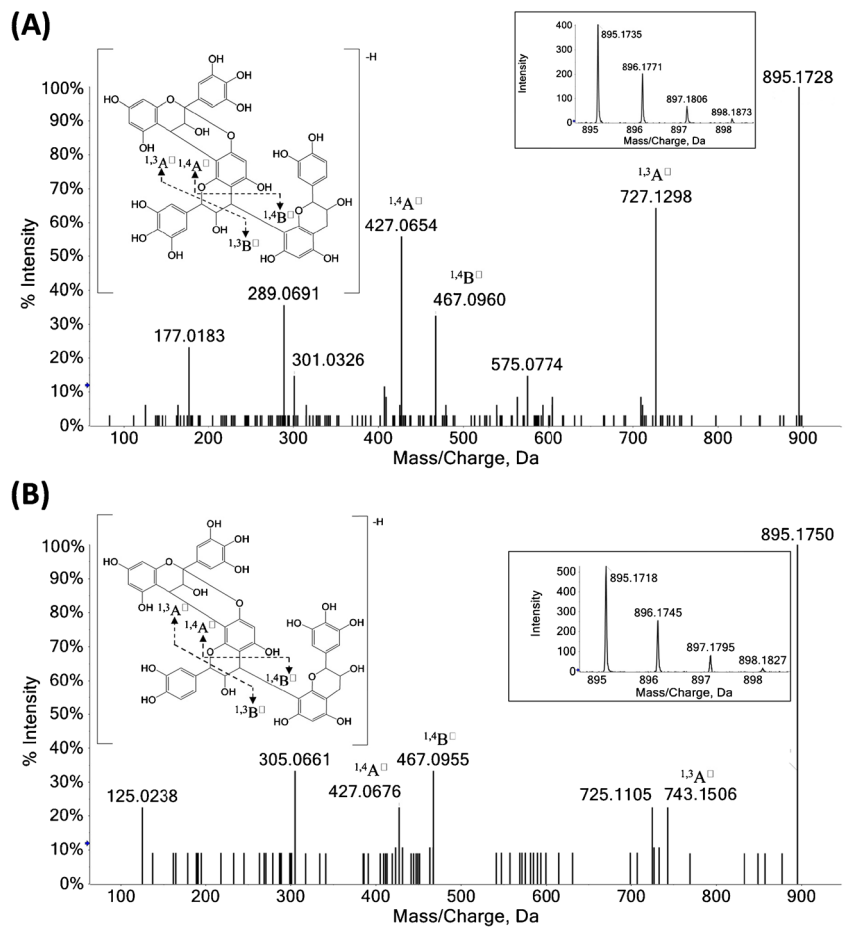


whereas only the last isomer was detected in *V. myrtillus* fruits. The presence of cinchonain isomers was previously highlighted in other plants [49], as well as in the leaves of various *Vaccinium* plant species [44, 50], but never observed before in berries.

**Comparison of polyphenolic compositions by PCA**

The polyphenolic compositions of the three investigated berry species, as reported in Tables 1, 2, 3, and 4 and discussed in the previous paragraphs, appear very complex. Therefore, PCAs were separately performed on the LC-ESI-TOF MS data acquired in positive and negative ionization in order to highlight which of the identified polyphenols are the most representative for describing the composition of the three *Vaccinium* species under investigation.

As regards positive ionization, two PCs had eigenvalues higher than 1 and were therefore considered as significant for describing the variance of the original TOF data. These two latent variables explained together 98.6% of the original variance (Fig. 4A). PC1, which accounted for 66.5% of the original variance, was positively and strongly correlated with coumaroyl-hexosides (e.g., peaks 51, 57, 58, and 63) and glucuronides (e.g., peaks 7 and 15), as well as with malvidin-feruloyl-

hexosides (peaks 61 and 64) and various anthocyanidin glycosides, such as dihexosides (e.g., peaks 3, 5, and 13) and aldopentose-hexosides (e.g., peaks 21 and 27). An opposite behavior (i.e., strong and negative correlation with PC1) was observed for malonyl (e.g., peak 39) and acetyl (e.g., peaks 49, 50, and 52) derivatives of anthocyanidins, as well as for various malvidin glycosides (e.g., peaks 29 and 36). Conversely, these last metabolites showed high and positive loadings on PC2 (explained variance equal to 32.1%), which was on the other hand negatively correlated with the five xyloside derivatives herein identified (i.e., peaks 24, 35, 37, 44, and 45). A very high and negative loading on PC2 was also observed for malvidin-3-*O*-glucoside (peak 34).

Figure 4B illustrates how the three analyzed samples of each species and the quality control samples (obtained by mixing equal amounts of each extracted sample) were located in the PC1 versus PC2 Cartesian plane. It is remarkable that different samples of each species were very close to the other, generating three well-separated clusters in the PC space. Accordingly, the repeatability of the whole analytical process as well as the robustness of the chemometric approach was demonstrated. It should also be noted that the quality controls were very close to the origin of the PC coordinates, confirming the accuracy and precision of PCA. The clusterization of the three *Vaccinium*

species clearly highlighted their very different whole anthocyanin compositions. More in detail, an important role in the discrimination of *V. myrtillus* samples, which showed very high and positive scores on PC1 and small and positive scores on PC2, was clearly played by the aforementioned coumaroyl-hexosides and glucuronides. Conversely, acetyl and malonyl derivatives were the major responsible for the separation of *V. corymbosum* fruits. Finally, *V. uliginosum* subsp. *gaultherioides* berries, even though generally poorer in the number of identified anthocyanins, as well as in their signal intensity, were interestingly characterized by xyloside derivatives of petunidin (peak 37), peonidin (peak 44), and malvidin (peak 45).

PCA was also applied to the TOF data acquired in negative mode, highlighting two factors with eigenvalues higher than 1, which accounted for a total explained variance of 96.4% (66.3% and 30.1% for PC1 and PC2, respectively). The variable separation on the two PCs was in this case not as good as that obtained for compounds detected under positive ionization, probably also due to the much higher number of analytes detected in negative polarity. In fact, many metabolites were distributed in a very wide range of negative PC1 values, with both positive and negative loadings on PC2. However, some analytes showed very high and positive loadings on PC1 and a very narrow range of PC2 values, thus forming a cluster (see Fig. 5A and the zoomed area). This cluster contained all the identified flavonol acetyl-hexoses (e.g., peaks 89 and 105), some flavonol aldopentosides (e.g., peaks 69, 77, and 87), and a number of B-type proanthocyanidins eluting at relatively low retention times, whereas no A/B-type derivatives were found in this group. Furthermore, some hydroxycinnamic acids, such as neochlorogenic (peak 175), cryptochlorogenic (peak 182), and coumaroylquinic (peak 177), belonged to the cluster. Cinchonains exhibited high loadings on PC1 and low loadings on PC2, as well. Conversely, very high and negative values on the former latent variable were observed for all flavonol glucuronides (e.g., peaks 65, 73, 83, 90, 106, and 111).

The score plot (Fig. 5B) highlighted a very good accuracy and precision of PCA also for data obtained under negative ionization, with quality control samples well centered on the origin of the PC coordinates and evident separations among *V. corymbosum* (high scores on PC1 and close to zero on PC2), *V. myrtillus* (high and negative scores on PC1 and very high and positive on PC2), and *V. uliginosum* subsp. *gaultherioides* (negative scores on both PC1 and PC2) berries. Thus, LC-ESI-MS/MS in negative ionization gave useful and complementary information with respect to the positive mode for the discrimination of the investigated species.

## Conclusions

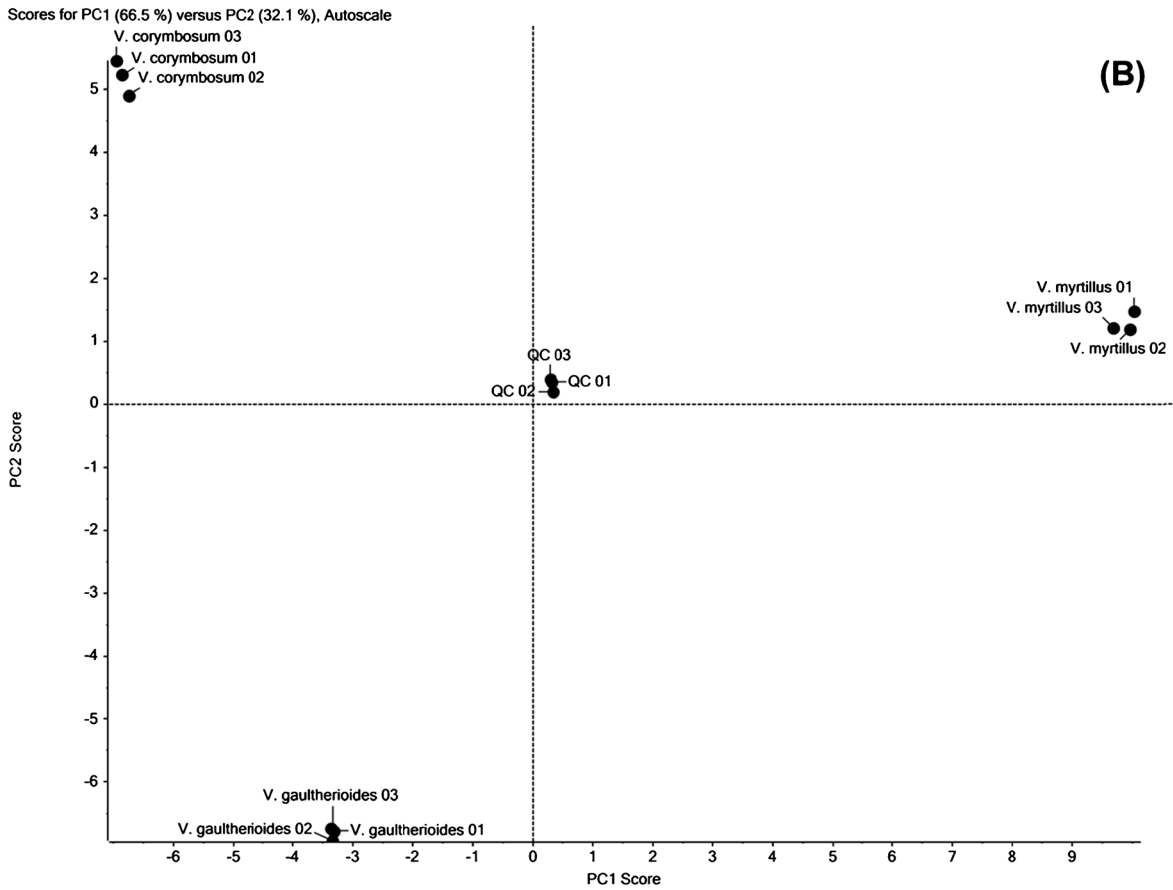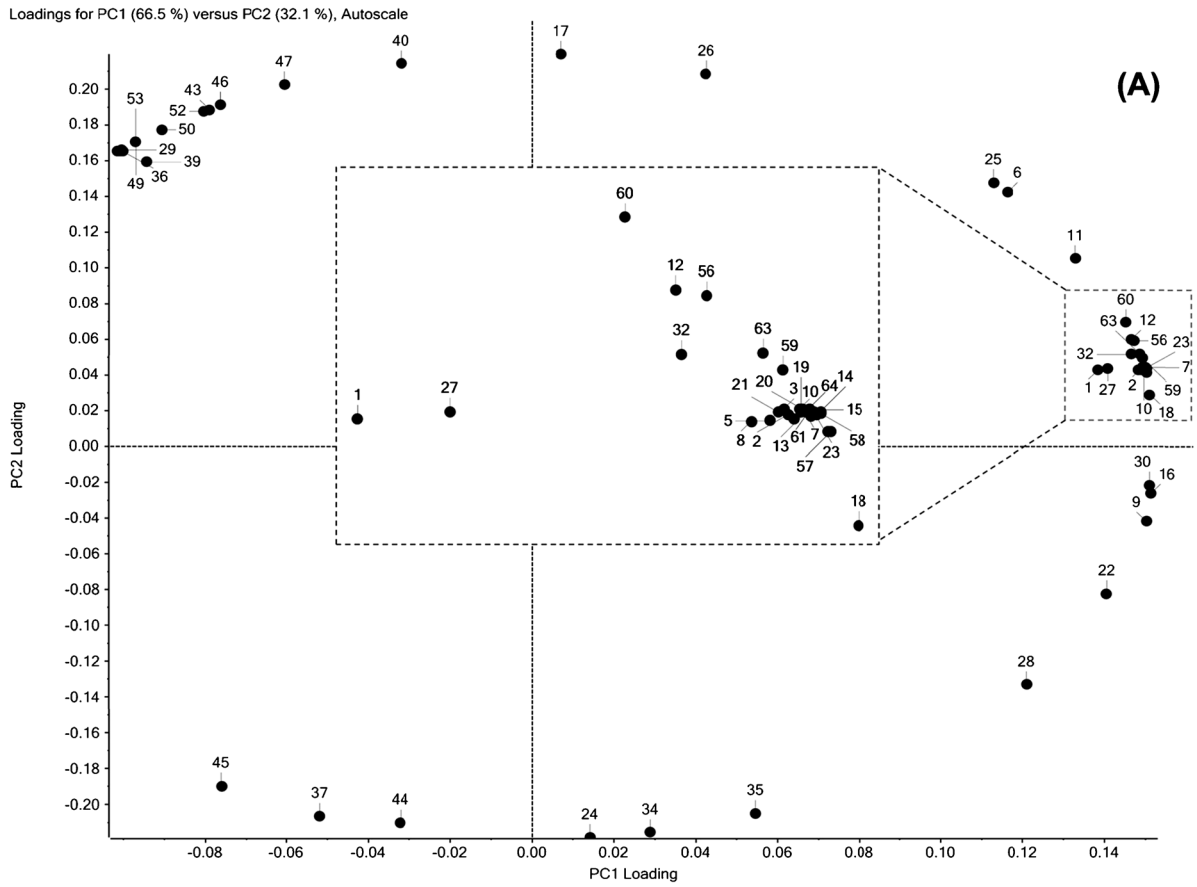LC-ESI-TOF and LC-ESI-Q/TOF analysis, performed both in positive and negative modes, allowed to obtain a comprehensive picture of the polyphenolic composition of *V. myrtillus*, *V. corymbosum*, and *V. uliginosum* subsp. *gaultherioides* berries.

More in detail, 58 of the 64 anthocyanins identified in this study were present in *V. myrtillus*, 39 in *V. corymbosum*, and 24 in *V. uliginosum* L. subsp. *gaultherioides*. As regards this last species, it is remarkable that aldopentosides and coumaroyl-hexosides have been detected herein for the first time. It should also be underlined that this study is the first one reporting the occurrence in *V. myrtillus* berries of anthocyanidin glucuronides and malvidin-feruloyl-hexosides, which represented an intense and characteristic metabolomic trait of this *Vaccinium* species, together with the already reported aldopentose-hexosides and cyanidin-aldodipentoside (Table S2). This study also indicated the exclusive presence of acetyl- and malonyl-hexosides in *V. corymbosum* berries (Table S2), compared to the other two investigated *Vaccinium* species.

Flavonols resulted generally more abundant in *V. corymbosum*. In fact, 51 of the 55 flavonols identified herein were found to be present in blueberries whereas only 37 in "false bilberry" and 35 in bilberry. Remarkably, in previous works, the flavonol derivatives discussed above were only partially detected in *V. myrtillus* [12, 13, 44] and *V. corymbosum* [24, 51, 52] berries, whereas very few data were elsewhere reported for *V. uliginosum* L. subsp. *gaultherioides* [4]. Hence, this work represents also for flavonol glycosides a more comprehensive study of such metabolites in the investigated *Vaccinium* species.

A similar number of flavanols were identified in the three species (i.e., 41, 39, and 35 compounds in bilberry, "false bilberry," and blueberry, respectively), and some of them, including trimers, tetramers, and pentamers, were found to be present in all species. However, some species-specific metabolites were found. For instance, flavanols containing A-type interflavanyl linkages were never observed in *V. corymbosum* (Table S2). Moreover, some B-type trimers (peaks 129, 144, and 157), tetramers (peaks 127, 130, 138, and 147), and pentamers (peaks 143, 146, 150, and 158) were exclusively found in blueberry (see Table 3). Interestingly, these compounds eluted at earlier retention times, compared to the metabolites common to the three species, thus suggesting a greater presence of catechin and gallocatechin, rather than the corresponding epimers, in *V. corymbosum* berries. It should be underlined that, for the first time, this research provides in-depth data on flavanols in *V. uliginosum* subsp. *gaultherioides* and *V. corymbosum* berries. Furthermore, even though data regarding flavanols in *V. myrtillus* fruits have been already

**Fig. 4** Loading (**A**) and score (**B**) plots of PC1 versus PC2 (PCA of original LC-ESI-TOF MS data acquired in positive ionization). *Numbers* shown in the loading plot refer to the peak numbers reported in Table 1

Loadings for PC1 (66.5 %) versus PC2 (32.1 %), Autoscale

**(A)**



Scores for PC1 (66.5 %) versus PC2 (32.1 %), Autoscale

**(B)**

◀ **Fig. 5** Loading (**A**) and score (**B**) plots of PC1 versus PC2 (PCA of original LC-ESI-TOF MS data acquired in negative ionization). *Numbers* shown in the loading plot refer to the peak numbers reported in Tables 2, 3, and 4

reported in literature [13, 44], this study provides a much more detailed description of the flavanol composition in these berries, identifying for the first time a large number of proanthocyanidins with high molecular weight.

All the aforementioned LC-MS data were well-integrated using the PCA approach, which demonstrated to be suitable for a clear discrimination of the investigated berry species both in positive and negative ionization modes.

The comprehensive investigation herein illustrated, which evidenced phenolic metabolites exclusively detected in one species or characterized by extremely different intensities in the three berries, can be useful for future developments of methods aiming at evaluating the quality of *Vaccinium* berry transformation products and to avoid frauds. These products, in fact, are not only fruit juices or jams that are not subjected to any particular regulation concerning their phenolic content but also supplements or actual drugs, which must conversely respect what is written in the label, both in terms of plant material used for its preparation and amount of active ingredients contained in the product.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

# References

1. Paredes-López O, Cervantes-Ceja M, Vigna-Pérez M, Hernández-Pérez T. Berries: improving human health and healthy aging, and promoting quality life—a review. Plant Foods Hum Nutr. 2010;65(3):299–308.

2. Ignat I, Volf I, Popa VI. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chem. 2011;126(4):1821–35.

3. Daglia M. Polyphenols as antimicrobial agents. Curr Opin Biotechnol. 2012;23(2):174–81.

4. Ancillotti C, Ciofi L, Pucci D, Sagona E, Giordani E, Biricolti S, et al. Polyphenolic profiles and antioxidant and antiradical activity of Italian berries from Vaccinium myrtillus L. and Vaccinium uliginosum L. subsp. gaultherioides (Bigelow) S.B. Young. Food Chem. 2016;204:176–84.

5. Määttä-Riihinen KR, Kamal-Eldin A, Mattila PH, González-Paramás AM, Törrönen AR. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. J Agric Food Chem. 2004;52(14):4477–86.

6. Beccaro G, Mellano MG, Botta R, Chiabrando V, Bounous G, editors. Phenolic and anthocyanin content and antioxidant activity in fruits of bilberry (Vaccinium myrtillus L.) and of highbush blueberry (V. corymbosum L.) cultivars in north Western Italy. Leuven: International Society for Horticultural Science (ISHS); 2006.

7. Može Š, Polak T, Gašperlin L, Koron D, Vanzo A, Poklar Ulrih N, et al. Phenolics in Slovenian bilberries (Vaccinium myrtillus L.) and blueberries (Vaccinium corymbosum L.). J Agric Food Chem. 2011;59(13):6998–7004.

8. Lätti AK, Riihinen KR, Kainulainen PS. Analysis of anthocyanin variation in wild populations of bilberry (Vaccinium myrtillus L.) in Finland. J Agric Food Chem. 2008;56(1):190–6.

9. Jovančević M, Balijagić J, Menković N, Šavikin K, Zdunić G, Janković T, et al. Analysis of phenolic compounds in wild populations of bilberry (Vaccinium myrtillus L.) from Montenegro. J Med Plant Res. 2011;5(6):910–4.

10. Giovanelli G, Buratti S. Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. Food Chem. 2009;112(4):903–8.

11. Åkerström A, Jaakola L, Bång U, Jäderlund A. Effects of latitude-related factors and geographical origin on anthocyanidin concentrations in fruits of Vaccinium myrtillus L. (bilberries). J Agric Food Chem. 2010;58(22):11939–45.

12. Laaksonen O, Sandell M, Kallio H. Chemical factors contributing to orosensory profiles of bilberry (Vaccinium myrtillus) fractions. Eur Food Res Technol. 2010;231(2):271–85.

13. Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Stampar F, Veberic R. A comparison of fruit quality parameters of wild bilberry (Vaccinium myrtillus L.) growing at different locations. J Sci Food Agric. 2015;95(4):776–85.

14. Gavrilova V, Kajdžanoska M, Gjamovski V, Stefova M. Separation, characterization and quantification of phenolic compounds in blueberries and red and black currants by HPLC–DAD–ESI-MSn. J Agric Food Chem. 2011;59(8):4009–18.

15. Del Bubba M, Checchini L, Chiuminatto U, Doumett S, Fibbi D, Giordani E. Liquid chromatographic/electrospray ionization tandem mass spectrometric study of polyphenolic composition of four cultivars of Fragaria vesca L. berries and their comparative evaluation. J Mass Spectrom. 2012;47(9):1207–20.

16. Sun J, Liu X, Yang T, Slovin J, Chen P. Profiling polyphenols of two diploid strawberry (Fragaria vesca) inbred lines using UHPLC-HRMS(n). Food Chem. 2014;146:289–98.

17. Ieri F, Martini S, Innocenti M, Mulinacci N. Phenolic distribution in liquid preparations of Vaccinium myrtillus L. and Vaccinium vitis idaea L. Phytochem Anal. 2013;24(5):467–75.

18. Ramirez JE, Zambrano R, Sepúlveda B, Kennelly EJ, Simirgiotis MJ. Anthocyanins and antioxidant capacities of six Chilean berries by HPLC–HR-ESI-ToF-MS. Food Chem. 2015;176:106–14.

19. Liu P, Lindstedt A, Markkinen N, Sinkkonen J, Suomela J-P, Yang B. Characterization of metabolite profiles of leaves of bilberry (Vaccinium myrtillus L.) and lingonberry (Vaccinium vitis-idaea L.). J Agric Food Chem. 2014;62(49):12015–26.

20. van der Hooft JJ, Vervoort J, Bino RJ, Beekwilder J, de Vos RC. Polyphenol identification based on systematic and robust high-resolution accurate mass spectrometry fragmentation. Anal Chem. 2010;83(1):409–16.

21. Beccaro GL, Giongo L, De Salvador FR, Ughini V, Folini L, Draicchio P, et al. Scegliere le cultivar di lampone, mirtillo e rovo per il 2011. L'Informatore Agrario. 2011;20:58–61 (In Italian).

22. Doumett S, Fibbi D, Cincinelli A, Giordani E, Nin S, Del Bubba M. Comparison of nutritional and nutraceutical properties in cultivated fruits of Fragaria vesca L. produced in Italy. Food Res Int. 2011;44(5):1209–16.

23. Barnes JS, Nguyen HP, Shen S, Schug KA. General method for extraction of blueberry anthocyanins and identification using high performance liquid chromatography–electrospray ionization-ion trap-time of flight-mass spectrometry. J Chromatogr A. 2009;1216(23):4728–35.

24. Sun J, Lin L, Chen P. Study of the mass spectrometric behaviors of anthocyanins in negative ionization mode and its applications for characterization of anthocyanins and non-anthocyanin polyphenols. Rapid Commun Mass Spectrom. 2012;26(9):1123–33.

25. Lätti AK, Riihinen KR, Jaakola L. Phenolic compounds in berries and flowers of a natural hybrid between bilberry and lingonberry (Vaccinium × intermedium Ruthe). Phytochemistry. 2011;72(8):810–5.

26. Veberic R, Slatnar A, Bizjak J, Stampar F, Mikulic-Petkovsek M. Anthocyanin composition of different wild and cultivated berry species. LWT Food Sci Technol. 2015;60(1):509–17.

27. Oliveira M, Esperanca P, Ferreira A. Characterisation of anthocyanidins by electrospray ionisation and collision-induced dissociation tandem mass spectrometry. Rapid Commun Mass Spectrom. 2001;15(17):1525–32.

28. Giusti MM, Rodríguez-Saona LE, Griffin D, Wrolstad RE. Electrospray and tandem mass spectroscopy as tools for anthocyanin characterization. J Agric Food Chem. 1999;47(11):4657–64.

29. Wu X, Prior RL. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. J Agric Food Chem. 2005;53(7):2589–99.

30. Felgines C, Talavera S, Texier O, Gil-Itsquierdo A, Lamaison JL, Remesy C. Blachberry anthocyanins are mainly recovered from urine as methylated and glucuronidated conjugates in humans. J Agric Food Chem. 2005;53(20):7721–7.

31. Ferrars R, Czank C, Zhang Q, Botting N, Kroon P, Cassidy A, et al. The pharmacokinetics of anthocyanins and their metabolites in humans. Brit J Pharmacol. 2014;171(13):3268–82.

32. Du Q, Jerz G, Winterhalter P. Isolation of two anthocyanin sambubiosides from bilberry (Vaccinium myrtillus) by high-speed counter-current chromatography. J Chromatogr A. 2004;1045(1):59–63.

33. Paes J, Dotta R, Barbero GF, Martínez J. Extraction of phenolic compounds and anthocyanins from blueberry (Vaccinium myrtillus L.) residues using supercritical CO2 and pressurized liquids. J Supercrit Fluids. 2014;95:8–16.

34. Lätti AK, Jaakola L, Riihinen KR, Kainulainen PS. Anthocyanin and flavonol variation in bog bilberries (Vaccinium uliginosum L.) in Finland. J Agric Food Chem. 2010;58(1):427–33.

35. Zoratti L, Jaakola L, Häggman H, Giongo L. Anthocyanin profile in berries of wild and cultivated Vaccinium spp. along altitudinal gradients in the Alps. J Agric Food Chem. 2015;63(39):8641–50.

36. He J-J, Liu Y-X, Pan Q-H, Cui X-Y, Duan C-Q. Different anthocyanin profiles of the skin and the pulp of Yan73 (Muscat Hamburg × Alicante Bouschet) grape berries. Molecules. 2010;15(3):1141–53.

37. Cuyckens F, Claeys M. Mass spectrometry in the structural analysis of flavonoids. J Mass Spectrom. 2004;39(1):1–15.

38. Lu L, Song FR, Tsao R, Jin YR, Liu ZQ, Liu SY. Studies on the homolytic and heterolytic cleavage of kaempferol and kaempferide glycosides using electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom. 2010;24(1):169–72.

39. Justesen U. Collision-induced fragmentation of deprotonated methoxylated flavonoids, obtained by electrospray ionization mass spectrometry. J Mass Spectrom. 2001;36(2):169–78.

40. Domon B, Costello CE. A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. Glycoconj J. 1988;5(4):397–409.

41. Ma Y, Li Q, Van den Heuvel H, Claeys M. Characterization of flavone and flavonol aglycones by collision-induced dissociation tandem mass spectrometry. Rapid Commun Mass Spectrom. 1997;11(12):1357–64.

42. Cuyckens F, Claeys M. Determination of the glycosylation site in flavonoid mono-O-glycosides by collision-induced dissociation of electrospray-generated deprotonated and sodiated molecules. J Mass Spectrom. 2005;40(3):364–72.

43. Petsalo A, Jalonen J, Tolonen A. Identification of flavonoids of Rhodiola rosea by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2006;1112(1):224–31.

44. Hokkanen J, Mattila S, Jaakola L, Pirttilä AM, Tolonen A. Identification of phenolic compounds from lingonberry (Vaccinium vitis-idaea L.), bilberry (Vaccinium myrtillus L.) and hybrid bilberry (Vaccinium x intermedium Ruthe L.) leaves. J Agric Food Chem. 2009;57(20):9437–47.

45. Gómez-Romero M, Zurek G, Schneider B, Baessmann C, Segura-Carretero A, Fernández-Gutiérrez A. Automated identification of phenolics in plant-derived foods by using library search approach. Food Chem. 2011;124(1):379–86.

46. Simirgiotis MJ, Theoduloz C, Caligari PD, Schmeda-Hirschmann G. Comparison of phenolic composition and antioxidant properties of two native Chilean and one domestic strawberry genotypes. Food Chem. 2009;113(2):377–85.

47. Teixeira N, Azevedo J, Mateus N, de Freitas V. Proanthocyanidin screening by LC–ESI-MS of Portuguese red wines made with teinturier grapes. Food Chem. 2016;190:300–7.

48. Jensen HD, Krogfelt KA, Cornett C, Hansen SH, Christensen SB. Hydrophilic carboxylic acids and iridoid glycosides in the juice of American and European cranberries (Vaccinium macrocarpon and V. oxycoccos), lingonberries (V. vitis-idaea), and blueberries (V. myrtillus). J Agric Food Chem. 2002;50(23):6871–4.

49. Nonaka G, Nishioka I. Tannins and related compounds. VII. Phenylpropanoid-substituted epicatechins, cinchonains from Cinchona succirubra. Chem Pharm Bull. 1982;30:4268–76.

50. Matsuo Y, Fujita Y, Ohnishi S, Tanaka T, Hirabaru H, Kai T, et al. Chemical constituents of the leaves of rabbiteye blueberry (Vaccinium ashei) and characterisation of polymeric proanthocyanidins containing phenylpropanoid units and A-type linkages. Food Chem. 2010;121(4):1073–9.

51. Cho MJ, Howard LR, Prior RL, Clark JR. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. J Sci Food Agric. 2004;84(13):1771–82.

52. Vrhovsek U, Masuero D, Palmieri L, Mattivi F. Identification and quantification of flavonol glycosides in cultivated blueberry cultivars. J Food Compos Anal. 2012;25(1):9–16.

## AUTHOR QUERIES

**AUTHOR PLEASE ANSWER ALL QUERIES.**

Q1.  Please check the suggested running page title if appropriate.
Q2.  Please check modifications made to Tables 2–4 if appropriate.