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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 <i>Vaccinium</i> species (i.e., <i>Vaccinium myrtillus</i> , <i>Vaccinium uliginosum</i> subsp. <i>gaultherioides</i> , and <i>Vaccinium corymbosum</i>). 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 <i>V. myrtillus</i> , or anthocyanidin aldopentosides and coumaroyl-hexosides in <i>V. uliginosum</i> subsp. <i>gaultherioides</i> 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 <i>Vaccinium</i> species investigated, on the basis of their polyphenolic composition, underlying one more time the fundamental role of mass spectrometry for food characterization.	
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Electronic supplementary material

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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 Ancillotti¹ · Lorenzo Ciofi¹ · Daniele Rossini¹ · Ugo Chiuminatto² · Jianru Stahl-Zeng² · Serena Orlandini¹ · Sandra Furlanetto¹ · Massimo Del Bubba¹

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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 anthocyanidin 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.

Keywords Polyphenols · Flavonoids · *Vaccinium* species · Liquid chromatography · High-resolution mass spectrometry · Principal component analysis

Introduction 40

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

Electronic supplementary material The online version of this article (doi:10.1007/s00216-016-0067-y) contains supplementary material, which is available to authorized users.

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

76 Furthermore, the feasibility of using the anthocyanin pro-
77 file as a species fingerprint becomes noteworthy for discern-
78 ing *V. myrtillus* from *V. uliginosum* L. subsp. *gaultherioides*.
79 In fact, the phenotype of this latter berry is very similar to the
80 *V. myrtillus* one, and the two fruits might be confused by the
81 harvesters involved in the production chain of transformed
82 bilberry. Italian *V. uliginosum* L. subsp. *gaultherioides* fruits
83 were recently analyzed for the first time by our team, evidenc-
84 ing a lower content of total soluble polyphenols and total
85 monomeric anthocyanins, as well as smaller antioxidant and
86 antiradical activities, compared to *V. myrtillus* ones [4]. Hence,
87 from this point of view, a lower nutraceutical value of “false
88 bilberry,” compared to bilberry, can be assumed. Concentra-
89 tions of individual anthocyanins found in “false bil-
90 berry” were in most cases lower than those of bilberry, as well.
91 Moreover, the relative abundance of the predominant antho-
92 cyanins of *V. uliginosum* L. subsp. *gaultherioides* berries was
93 found very different from that of *V. myrtillus* fruits [4] and,
94 interestingly, rather similar to the profile of *V. corymbosum*,
95 being for instance both “false bilberry” and blueberry charac-
96 terized by the predominance of malvidin derivatives [4, 7].

97 The analysis of further classes of polyphenols, such as fla-
98 vonols, flavanols, and phenolic acids, which might be also
99 important for discriminating one *Vaccinium* species from an-
100 other, has been performed only occasionally in *V. myrtillus*
101 [12, 13] and *V. corymbosum* berries [14]. Data concerning
102 some phenolic compounds have been recently reported also
103 for *V. uliginosum* L. subsp. *gaultherioides* berries [4].

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

108 In order to carry out such a kind of studies, complex ana-
109 lytical approaches, involving nontarget metabolomic investi-
110 gations, are required. These investigations are commonly per-
111 formed using liquid chromatography (LC) coupled with mass
112 spectrometry (MS) [15, 16], employing in some cases also
113 ultraviolet detection [17, 18] and occasionally nuclear mag-
114 netic resonance, as well [19]. Actually, LC-MS is one of the
115 most powerful analytical technique for polyphenol analysis.
116 In fact, atmospheric pressure ionization sources provide a soft
117 ionization of target analytes, which is particularly recom-
118 mended for structure elucidation of polar, nonvolatile, and
119 thermally labile compounds, such as flavonoids. Moreover,
120 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 adop-
tion of high-resolution mass spectrometry (e.g., time-of-flight-
based instruments) allows for obtaining accurate mass read-
out, thus facilitating the assignment of an elemental formula to
the parent molecule and/or to the fragments and its fragmen-
tation characteristics [20].

Based on the above-reported considerations, this study
aimed at comprehensively investigating the polyphenolic pro-
files 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 spec-
trometry (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, quercetin-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-
dicafeoylquinic 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* sam-
ples 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

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

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

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

182 After the sampling, all berries were immediately frozen in
183 liquid nitrogen, freeze-dried, and finally ground in order to
184 obtain a homogeneous powder. All samples were stored at
185 $-20\text{ }^{\circ}\text{C}$ until analyses were performed.

186 Sample extraction

187 Three representative aliquots from each berry sample were
188 extracted according to a procedure previously developed for
189 *Fragaria vesca* berries [22] and successively verified for bil-
190 berry and “false bilberry” [4]. Briefly, about 500 mg dry
191 weight (d.w.) berry aliquots were homogenized in an ice bath
192 under magnetic stirring with 15 mL of a methanol/water so-
193 lution 8/2 (v/v), containing NaF 10 mM to inactivate polyph-
194 enol oxidase; the mixture was centrifuged at $1800\times g$ for 5 min
195 and the supernatant recovered. This procedure was repeated
196 three times and the resulting extracts were combined. The
197 organic solvent was removed by vacuum evaporation, acidi-
198 fied with formic acid up to $\text{pH}=2.0\pm 0.1$ (volume of formic
199 acid 170–190 μL), and filtrated at 0.2 μm with nylon mem-
200 branes, before LC-MS/MS analysis. A final extract volume of
201 approximately 9.2 mL was therefore obtained.

202 LC-TOF and LC-Q/TOF analysis

203 LC analysis was performed on an Agilent Infinity 1290 sys-
204 tem equipped with an Acquity BEH C18 column
205 (15 cm \times 2.1 cm i.d., particle size 1.7 μm) and a guard column
206 containing the same stationary phase (Waters, Milford, MA,
207 USA). Column temperature was set at 50 $^{\circ}\text{C}$. Water/formic
208 acid 95:5 v/v (eluent A) and methanol/formic acid 95:5 v/v
209 (eluent B) were used for the analyte elution, according to the
210 following gradients: 0–2 min isocratic 2% B, 2–30 min linear
211 gradient 2–30% B, 30–35 min linear gradient 30–95% B, and
212 35–37 min isocratic 95% B. The flow rate was 450 $\mu\text{L}/\text{min}$
213 and the injection volume was 2 μL .

214 The LC system was coupled with a SCIEX (Framingham,
215 MA, USA) TripleTOF[®] 5600 hybrid Q/TOF mass analyzer
216 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 $^{\circ}\text{C}$, 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 nega-
tive 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 candi-
date ions collected within each cycle, by the Information
Dependent Acquisition (IDA) software. Q/TOF MS/MS spec-
tra 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 $\pm 15\text{ eV}$ (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 (accu-
mulation time of 50 ms and whole cycle time 800 ms).

Automated calibration was performed using an external
calibrant delivery system (CDS) which infuses proper calibra-
tion 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 nega-
tive and positive ionization, needs to be processed with spe-
cific software. PeakView[®] 2.2 and MasterView[®] 1.1 soft-
ware 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: $<5\text{ ppm}$
- Isotope ratio difference compared to the theoretical iso-
tope profile: $<20\%$
- Purity score of the MS/MS spectra compared to the one of
available standards: $\geq 80\%$

In this manuscript, we used the words “identification/iden-
tified,” sometimes stressed by the words “undoubted/un-
doubtedly,” “unequivocal/unequivocally” when an authentic
reference standard was available. Conversely, the terms “pu-
tative/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 polyph-
enols that mainly contributed to their differentiation, principal

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

275 Results and discussion

276 Compound identification by LC-ESI-TOF 277 and LC-ESI-Q/TOF analysis

278 The polyphenols found in berries of the investigated
279 *Vaccinium* species were identified according to their chro-
280 matographic behavior, their TOF MS and Q/TOF MS/MS
281 spectra, also in comparison with standard reference com-
282 pounds, when available. Both positive and negative ionization
283 modes were used for compound attribution.

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

289 Compound identification within each class is detailed be-
290 low and summarized in Tables 1, 2, 3, and 4, which show
291 retention time (Rt, min), mass (Da) found by the TOF survey
292 scan experiment and main MS/MS fragments (Da) obtained
293 by the Q/TOF experiment, proposed formula and correspond-
294 ing exact mass (Da), mass accuracy (Δ , ppm), and putative
295 identification of the peaks considered. Peaks reported in these
296 tables were also shown in Figs. S1–S4 of the Electronic sup-
297plementary material (ESM).

298 Anthocyanins

299 Anthocyanins are characterized by a positive charge at pH < 3
300 and therefore are typically determined in the form of molecu-
301 lar ion $[M]^+$ [23]; accordingly, these polyphenols were identi-
302 fied under positive ionization (Table 1). Moreover, their attri-
303 bution was also confirmed under negative ionization, by moni-
304 toring the quasi-molecular ion $[M-2H]^-$, according to the
305 mass spectrometric behavior observed for these polyphenols
306 by Sun and colleagues [24]. However, for this latter ionization
307 mode, a less complete profile of the anthocyanin fraction was
308 obtained, owing to its generally lower sensitivity that
309 prevented in several cases the signal detection (data not
310 shown).

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

delphinidin vs. quercetin, cyanidin vs. kaempferol, and 313
petunidin vs. isorhamnetin derivatives of the same sugar), 314
the $[M]^+$ or the $[M-2H]^-$ ions of the former and the $[M+H]^+$ 315
or the $[M-H]^-$ ions of the latter are isobars, thus making rel- 316
evant for their discrimination the chromatographic behavior. 317

As widely reported elsewhere [15, 18, 25], also in this 318
study, MS/MS fragmentation of anthocyanins produced only 319
the loss of the sugar units (e.g., 162 Da for a hexose and 320
132 Da for a pentose) and the corresponding detection of the 321
aglycone fragment (i.e., 287.06 Da for cyanidin, 303.05 Da 322
for delphinidin, 317.07 Da for petunidin, 301.07 Da for 323
peonidin, and 331.08 Da for malvidin) (Table 1). 324

Using the IDA TOF-Q/TOF workflow and, when possi- 325
ble, by comparing the retention time and mass spectra 326
of unknowns with those of authentic standards, the un- 327
doubted or at least the tentative identification of 64 328
anthocyanins was achieved. TOF MS $[M]^+$ molecular 329
ions matched the proposed formulae with very high 330
mass accuracy, being Δ absolute values ≤ 1 ppm in 331
about 80% of the cases, and included in the range of 332
1.1–2.2 ppm for the remaining compounds (Table 1). 333
Among the 64 anthocyanins identified, the presence of 334
the 3-*O*-glucoside derivatives of delphinidin, cyanidin, 335
petunidin, peonidin, and malvidin (peaks 9, 16, 22, 28, 336
and 34); 3-*O*-galactoside derivatives of delphinidin, 337
cyanidin, peonidin, and malvidin (peaks 6, 12, 25, and 338
29); and 3-*O*-arabinoside derivatives of cyanidin and 339
peonidin (peaks 18 and 32) was confirmed by spiking 340
the extracts with authentic reference standards (Table 1). 341
Peaks 11, 17, 26, and 36 were putatively annotated to 342
delphinidin-3-*O*-arabinoside, petunidin-3-*O*-galactoside, 343
petunidin-3-*O*-arabinoside, and malvidin-3-*O*-arabinoside 344
on the basis of their (i) TOF MS accuracy, isotope ratio 345
difference, and MS/MS data (Y_0 cleavage of the sugar 346
and formation of the aglycone ion), as well as (ii) rel- 347
ative chromatographic retention, the latter being in 348
agreement with the retention observed by various au- 349
thors under reversed-phase conditions for different gly- 350
cosides with the same aglycone (i.e., increasing reten- 351
tion in the order galactoside < glucoside < arabinoside) 352
and for different anthocyanins glycosylated with the 353
same sugar (i.e., increasing retention in the order 354
delphinidin < cyanidin < petunidin < peonidin < malvidin) 355
[4, 7, 23]. The 15 anthocyanins reported above were 356
detected in all the investigated species and resulted in 357
all cases among the most abundant anthocyanidin deriv- 358
atives (signal intensities approximately included in be- 359
tween 1×10^5 and 1×10^6 counts), as widely reported 360
elsewhere [4, 7, 8, 26]. 361

Among the first eluting analytes (peaks 1–5, Rt = 9.7– 362
11.4 min), which were detected only in the *V. myrtillus* berry 363
extracts and with very low signal intensity (i.e., 1000–1800 364
counts), peaks 1, 3, and 5 exhibited an $[M]^+$ ion at 627.15 Da 365

Study and evaluation of the polyphenolic composition of *Vaccinium*

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 (Δ , 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

t1.2	Peak	Rt	TOF MS	Q/TOF MS/MS	Proposed formula	Exact mass	Δ	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 (III) ^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-aldodipentose ^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-aldopentose
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	Δ	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 Y₀ 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, m/z
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.,

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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

t2.2	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	<i>317.0297</i> ; 178.9989; 151.0036	C ₂₁ H ₁₈ O ₁₄	493.0624	−1.3	+	+	+	Myricetin-glucuronide
t2.4	66	19.9	479.0826	<i>317.0299</i> ; <i>316.0225</i> ; 271.0229	C ₂₁ H ₂₀ O ₁₃	479.0831	−1.1	+	+	+	Myricetin-3- <i>O</i> -galactoside
t2.5	67	20.6	479.0830	<i>317.0299</i> ; <i>316.0216</i> ; 271.0236	C ₂₁ H ₂₀ O ₁₃	479.0831	−0.1	+	+	+	Myricetin-3- <i>O</i> -glucoside
t2.6	68	20.9	449.0713	<i>317.0288</i> ; <i>316.0207</i> ; 271.0236	C ₂₀ H ₁₈ O ₁₂	449.0725	−2.8	+	+	−	Myricetin-3- <i>O</i> -aldopentoside (I)
t2.7	69	22.5	449.0714	<i>317.0281</i> ; <i>316.0212</i> ; 271.0230	C ₂₀ H ₁₈ O ₁₂	449.0725	−2.6	−	+	+	Myricetin-3- <i>O</i> -aldopentoside (II)
t2.8	70	22.9	449.0715	<i>317.0240</i> ; <i>316.0211</i> ; 271.0251	C ₂₀ H ₁₈ O ₁₂	449.0725	−2.4	+	+	+	Myricetin-3- <i>O</i> -aldopentoside (III)
t2.9	71	24.1	463.0876	<i>301.0346</i> ; <i>300.0271</i> ; 271.0248	C ₂₁ H ₂₀ O ₁₂	463.0882	−1.4	+	+	+	Quercetin-3- <i>O</i> -galactoside ^a
t2.10	72	24.2	521.0926	<i>317.0278</i> ; <i>316.0216</i> ; 271.0246	C ₂₃ H ₂₂ O ₁₄	521.0937	−2.1	−	−	+	Myricetin-acetyl-hexoside (I)
t2.11	73	24.4	477.0661	<i>301.0352</i> ; 178.9979; 151.0028	C ₂₁ H ₁₈ O ₁₃	477.0675	−2.8	+	+	+	Quercetin-glucuronide ^a
t2.12	74	24.6	609.1452	<i>301.0335</i> ; <i>300.0262</i> ; 271.0235	C ₂₇ H ₃₀ O ₁₆	609.1461	−1.5	+	−	+	Quercetin-3- <i>O</i> -deoxyhexose-hexoside
t2.13	75	25.0	463.0879	<i>301.0346</i> ; <i>300.0267</i> ; 271.0248	C ₂₁ H ₂₀ O ₁₂	463.0882	−0.7	+	+	+	Quercetin-3- <i>O</i> -glucoside ^a
t2.14	76	25.6	609.1454	<i>301.0335</i> ; <i>300.0255</i>	C ₂₇ H ₃₀ O ₁₆	609.1461	−1.2	−	−	+	Quercetin-3- <i>O</i> -rutinoside ^a
t2.15	77	25.7	433.0762	<i>301.0341</i> ; <i>300.2630</i> ; 271.0230	C ₂₀ H ₁₈ O ₁₁	433.0776	−3.3	+	+	+	Quercetin-3- <i>O</i> -aldopentoside (I)
t2.16	78	26.1	493.0983	<i>331.0450</i> ; <i>330.0369</i> ; 315.1320	C ₂₂ H ₂₂ O ₁₃	493.0988	−1.0	+	+	+	Laricitrin-3- <i>O</i> -galactoside
t2.17	79	26.4	433.0768	<i>301.0341</i> ; <i>300.0267</i> ; 271.0237	C ₂₀ H ₁₈ O ₁₁	433.0776	−1.9	+	+	+	Quercetin-3- <i>O</i> -aldopentoside (II)
t2.18	80	26.4	521.0941	<i>317.0271</i> ; <i>316.0202</i> ; 271.0225	C ₂₃ H ₂₂ O ₁₄	521.0937	0.7	−	−	+	Myricetin-acetyl-hexoside (II)
t2.19	81	26.5	317.0294	<i>178.9983</i> ; <i>165.0192</i> ; <i>151.0031</i> ; <i>137.0237</i>	C ₁₅ H ₁₀ O ₈	317.0303	−2.8	+	+	+	Myricetin ^a
t2.20	82	26.6	493.0989	<i>331.0461</i> ; <i>330.0383</i> ; 315.0151	C ₂₂ H ₂₂ O ₁₃	493.0988	0.3	+	+	+	Laricitrin-3- <i>O</i> -glucoside
t2.21	83	26.7	507.0777	<i>331.0445</i> ; 316.0210; 178.9978	C ₂₂ H ₂₀ O ₁₄	507.0780	−0.7	+	+	+	Laricitrin-glucuronide
t2.22	84	26.7	549.0876	<i>505.0983</i> ; <i>463.0870</i> ; <i>301.0333</i> ; <i>300.0263</i>	C ₂₄ H ₂₂ O ₁₅	549.0886	−1.8	−	−	+	Quercetin-malonyl-hexoside (I)
t2.23	85	27.0	549.0877	<i>505.0997</i> ; <i>463.0892</i> ; <i>301.0350</i> ; <i>300.0271</i>	C ₂₄ H ₂₂ O ₁₅	549.0886	−1.6	−	−	+	Quercetin-malonyl-hexoside (II)
t2.24	86	27.2	447.0912	<i>285.0395</i> ; <i>284.0308</i> ; <i>255.0280</i> ; <i>227.0335</i>	C ₂₁ H ₂₀ O ₁₁	447.0933	−3.2	+	+	+	Kaempferol-3- <i>O</i> -galactoside
t2.25	87	27.5	433.0768	<i>301.0343</i> ; <i>300.0270</i> ; 271.0236	C ₂₀ H ₁₈ O ₁₁	433.0776	−2.0	−	+	+	Quercetin-3- <i>O</i> -aldopentoside (III)
t2.26	88	28.0	463.0862	<i>331.0456</i> ; <i>330.0361</i> ; 315.0149	C ₂₁ H ₂₀ O ₁₂	463.0882	−4.4	+	+	+	Laricitrin-3- <i>O</i> -xyloside
t2.27	89	28.2	505.0972	<i>301.0338</i> ; <i>300.0273</i> ; 271.0237	C ₂₃ H ₂₂ O ₁₃	505.0988	−3.0	−	−	+	Quercetin-acetyl-hexoside (I)
t2.28	90	28.4	461.0721	<i>285.0390</i> ; <i>257.0438</i> ; 229.0491	C ₂₁ H ₁₈ O ₁₂	461.0725	−0.9	+	+	+	Kaempferol-glucuronide
t2.29	91	28.5	463.0869	<i>331.0431</i> ; <i>330.0378</i> ; 315.0140	C ₂₁ H ₂₀ O ₁₂	463.0882	−2.9	+	+	+	Laricitrin-3- <i>O</i> -aldopentoside (I)
t2.30	92	28.8	447.0923	<i>301.0350</i> ; <i>300.0271</i> ; 271.0238	C ₂₁ H ₂₀ O ₁₁	447.0933	−2.1	+	−	+	Quercetin-3- <i>O</i> -rhamnoside ^a
t2.31	93	28.9	447.0923	<i>285.0397</i> ; <i>284.0325</i> ; <i>255.0293</i> ; <i>227.0348</i>	C ₂₁ H ₂₀ O ₁₁	447.0933	−2.1	−	+	−	Kaempferol-3- <i>O</i> -glucoside
t2.32	94	29.3	417.0823	<i>284.0316</i> ; <i>255.0291</i> ; <i>227.0343</i>	C ₂₀ H ₁₈ O ₁₀	417.0827	−0.9	+	+	+	Kaempferol-3- <i>O</i> -aldopentoside
t2.33	95	29.3	505.0979	<i>301.0352</i> ; <i>300.0272</i> ; 271.0237	C ₂₃ H ₂₂ O ₁₃	505.0988	−1.6	−	−	+	Quercetin-acetyl-hexoside (II)
t2.34	96	29.5	593.1504	<i>285.0398</i> ; <i>284.0326</i>	C ₂₇ H ₃₀ O ₁₅	593.1512	−1.3	−	−	+	Kaempferol-7- <i>O</i> -neohesperidoside ^a
t2.35	97	29.6	477.1032	<i>314.0419</i> ; 271.0232; 243.0290	C ₂₂ H ₂₂ O ₁₂	477.1038	−1.4	+	+	+	Isorhamnetin-3- <i>O</i> -galactoside
t2.36	98	29.7	505.0977	<i>301.0337</i> ; <i>300.0268</i> ; <i>255.0293</i>	C ₂₃ H ₂₂ O ₁₃	505.0988	−2.1	−	−	+	Quercetin-acetyl-hexoside (III)
t2.37	99	30.0	463.0870	<i>330.0355</i> ; <i>331.0455</i> ; 315.0133	C ₂₁ H ₂₀ O ₁₂	463.0882	−2.5	−	+	+	Laricitrin-3- <i>O</i> -aldopentoside (II)
t2.38	100	30.0	579.1350	<i>301.0351</i> ; <i>300.0262</i> ; 271.0235	C ₂₆ H ₂₈ O ₁₅	579.1355	−0.9	+	−	−	Quercetin-3- <i>O</i> -deoxyhexose-pentoside
t2.39	101	30.2	623.1613	<i>315.0496</i> ; <i>314.0405</i> ; 299.0174	C ₂₈ H ₃₂ O ₁₆	623.1618	−0.8	−	−	+	Isorhamnetin-3- <i>O</i> -deoxyhexose-hexoside
t2.40	102	30.2	505.0983	<i>301.0335</i> ; <i>300.0255</i> ; 271.0226	C ₂₃ H ₂₂ O ₁₃	505.0988	−0.9	−	−	+	Quercetin-acetyl-hexoside (IV)
t2.41	103	30.4	477.1029	<i>314.0426</i> ; 271.0258; 243.0299	C ₂₂ H ₂₂ O ₁₂	477.1038	−2.0	+	+	−	Isorhamnetin-3- <i>O</i> -glucoside
t2.42	104	30.4	477.1029	<i>331.0438</i> ; <i>330.0362</i> ; 315.0135	C ₂₂ H ₂₂ O ₁₂	477.1038	−2.0	−	−	+	Laricitrin-3- <i>O</i> -rhamnoside
t2.43	105	30.6	535.1078	<i>330.0362</i> ; 315.097	C ₂₄ H ₂₄ O ₁₄	535.1093	−2.9	−	−	+	Laricitrin-acetyl-hexoside
t2.44	106	30.7	491.0833	<i>315.0509</i> ; <i>300.0255</i> ; 271.0239	C ₂₂ H ₂₀ O ₁₃	491.0831	0.4	+	+	+	Isorhamnetin-glucuronide
t2.45	107	30.8	507.1141	<i>345.0616</i> ; <i>344.0530</i> ; 301.0353	C ₂₃ H ₂₄ O ₁₃	507.1144	−0.7	+	+	+	Syringetin-3- <i>O</i> -galactoside

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

Peak	Rt	TOF MS	Q/TOF MS/MS	Proposed formula	Exact mass	Δ	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-aldopentose
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-aldopentose (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-aldopentose (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-aldopentose (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-aldopentose (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-aldopentose (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 were detected in berries
434 from all the investigated *Vaccinium* species (Table 1) and can
435 be ascribed to aldopentose 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 aldopentoses 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-aldopentose, 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

Study and evaluation of the polyphenolic composition of *Vaccinium*

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 (Δ , 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

t3.2	Peak	Rt	TOF MS	Q/TOF MS/MS	Proposed formula	Exact mass	Δ	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	+	+	+	Galocatechin
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)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)C-(E)GC-(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-(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-(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-(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-(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)C-(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 (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 (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)gallo catechin

^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

486 delphinidin (peaks 33 and 41), cyanidin (peaks 40 and 47),
 487 petunidin (peaks 43 and 50), peonidin (peaks 46 and 52), and
 488 malvidin (peaks 49 and 53). Interestingly, these acylated antho-
 489 cyanins showed much higher intensities in blueberry
 490 (1.0×10^4 – 1.4×10^5 counts) than in bilberry (4.0×10^3 –
 491 1.0×10^4 counts) [29], whereas they were never detected in
 492 false bilberry (Table 1), thus representing a potential group of
 493 markers for the differentiation of these fruit species.

494 Peaks 39, 42, and 48 were also characteristic of blueberry,
 495 which were absent in bilberries and “false bilberries.” The
 496 MS/MS spectra evidenced the presence of cyanidin and
 497 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 deriva-
 tives 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,

Study and evaluation of the polyphenolic composition of *Vaccinium*

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

t4.2	Peak	Rt	TOF MS	Q/TOF MS/MS	Proposed formula	Exact mass	Δ	M	G	C	Tentative identification
t4.3	174	2.0	169.0146	<i>125.0240</i> ; 124.0244; 79.0189	C ₇ H ₆ O ₅	169.0142	2.0	+	+	+	Gallic acid ^a
t4.4	175	6.8	353.0866	<i>191.0554</i> ; 179.0345; 135.0446	C ₁₆ H ₁₈ O ₉	353.0878	−3.4	+	+	+	Neochlorogenic acid ^a
t4.5	176	9.2	337.0918	<i>191.0562</i> ; <i>163.0390</i> ; 119.0499	C ₁₆ H ₁₈ O ₈	337.0929	−3.2	−	−	+	Coumaroylquinic acid (I)
t4.6	177	9.4	337.0916	<i>191.0554</i> ; <i>163.0387</i> ; 119.0505	C ₁₆ H ₁₈ O ₈	337.0929	−3.7	−	−	+	Coumaroylquinic acid (II)
t4.7	178	10.0	179.0351	<i>135.0444</i> ; 134.0368	C ₉ H ₈ O ₄	179.0350	0.6	+	+	+	Caffeic acid ^a
t4.8	179	10.6	341.0872	<i>179.0342</i> ; <i>135.0446</i> ; 134.0358	C ₁₅ H ₁₈ O ₉	341.0878	−1.7	+	+	+	Caffeic acid hexoside
t4.9	180	11.0	353.0869	<i>191.0566</i>	C ₁₆ H ₁₈ O ₉	353.0878	−2.6	+	+	+	Chlorogenic acid ^a
t4.10	181	12.3	325.0920	<i>163.0395</i> ; <i>119.0504</i>	C ₁₅ H ₁₈ O ₈	325.0929	−2.6	+	+	+	Coumaric acid hexoside
t4.11	182	12.4	353.0868	<i>191.0552</i> ; 179.0348; <i>173.0451</i> ; 135.0448	C ₁₆ H ₁₈ O ₉	353.0878	−2.8	+	+	+	Cryptochlorogenic acid ^a
t4.12	183	13.7	355.1031	193.0507; <i>175.0398</i> ; <i>160.0163</i> ; 132.0210	C ₁₆ H ₂₀ O ₉	355.1035	−1.0	+	+	+	Ferulic acid hexoside
t4.13	184	13.9	739.1657	<i>587.1205</i> ; 339.0494; 289.0707; 177.0190	C ₃₉ H ₃₂ O ₁₅	739.1668	−1.5	−	−	+	Cinchonain IIx (I)
t4.14	185	14.7	337.0918	<i>191.0547</i> ; 163.0384	C ₁₆ H ₁₈ O ₈	337.0929	−3.3	+	−	+	Coumaroylquinic acid (III)
t4.15	186	15.4	353.0873	<i>191.0568</i>	C ₁₆ H ₁₈ O ₉	353.0878	−1.4	+	−	+	Caffeoylquinic acid
t4.16	187	16.0	739.1658	<i>587.1192</i> ; 449.0864; 339.0498; <i>289.0704</i>	C ₃₉ H ₃₂ O ₁₅	739.1668	−1.5	−	−	+	Cinchonain IIx (II)
t4.17	188	16.2	335.0767	<i>179.0343</i> ; 161.0207; <i>135.0446</i> ; 134.0372	C ₁₆ H ₁₆ O ₈	335.0772	−1.6	+	+	+	Caffeic acid derivative
t4.18	189	16.9	739.1641	<i>587.1170</i> ; 339.0510; <i>289.0704</i>	C ₃₉ H ₃₂ O ₁₅	739.1668	−3.8	+	−	+	Cinchonain IIx (III)
t4.19	190	17.4	191.0349	<i>176.0109</i> ; <i>104.0277</i>	C ₁₀ H ₈ O ₄	191.0350	−0.5	−	+	−	Scopoletin ^a
t4.20	191	17.5	739.1667	<i>587.1195</i> ; 449.0871; 339.0484; <i>289.0688</i> ; <i>177.0174</i>	C ₃₉ H ₃₂ O ₁₅	739.1668	−0.2	+	−	+	Cinchonain IIx (IV)
t4.21	192	17.6	367.1025	193.0494; <i>191.0555</i> ; 173.0452; 134.0373	C ₁₇ H ₂₀ O ₉	367.1035	−2.6	+	+	+	Feruloylquinic acid
t4.22	193	17.9	193.0504	<i>134.0367</i> ; 133.0302	C ₁₀ H ₁₀ O ₄	193.0506	−1.3	−	−	+	Ferulic acid ^a
t4.23	194	21.2	367.1026	<i>179.0342</i> ; 161.0256 <i>135.0446</i> ; 134.0357	C ₁₇ H ₂₀ O ₉	367.1035	0.8	+	+	+	Caffeic acid derivative
t4.24	195	24.4	535.1454	371.0980; 329.1038; 191.0346; <i>163.0398</i> ; <i>147.0450</i> ; 119.0501	C ₂₅ H ₂₈ O ₁₃	535.1457	−0.5	+	+	−	Coumaroyl iridoid (I)
t4.25	196	25.7	535.1453	371.0987; 329.1025; 191.0337; <i>163.0396</i> ; <i>147.0443</i> ; 119.0500	C ₂₅ H ₂₈ O ₁₃	535.1457	−0.7	+	+	−	Coumaroyl iridoid (II)
t4.26	197	26.2	515.1184	<i>353.0867</i> ; <i>191.0555</i> ; <i>179.0345</i> ; 173.0452; 135.0450	C ₂₅ H ₂₄ O ₁₂	515.1195	−2.2	−	−	+	Dicaffeoylquinic acid
t4.27	198	26.8	515.1179	<i>353.0872</i> ; <i>191.0550</i> ; 179.0347; 135.0445	C ₂₅ H ₂₄ O ₁₂	515.1195	−3.0	−	−	+	1,5-Dicaffeoylquinic acid ^a
t4.28	199	28.0	435.1291	<i>273.0751</i> ; 167.0322	C ₂₁ H ₂₄ O ₁₀	435.1297	−1.3	+	+	+	Phloridzin ^a
t4.29	200	32.1	411.1659	163.0402; <i>145.0290</i> ; 119.0481	C ₂₀ H ₂₈ O ₉	411.1661	1.5	+	+	−	Coumaric acid-malonyl-hexoside (I)
t4.30	201	32.5	411.1659	<i>163.0398</i> ; <i>145.0292</i> ; 119.0498	C ₂₀ H ₂₈ O ₉	411.1661	−0.3	+	+	−	Coumaric acid-malonyl-hexoside (II)
t4.31	202	32.6	445.1143	<i>179.0346</i> ; <i>135.0441</i> ; 134.0367	C ₂₂ H ₂₂ O ₁₀	445.1040	0.6	+	+	+	Caffeic acid derivative

^a Confirmed by spiking the extracts with authentic standards

510 peaks 58 and 62), and *m/z* 331.08 (i.e., malvidin, peaks 60
 511 and 63). The signal intensities of these compounds were
 512 much higher in *V. myrtillus* berries (approximately from
 513 1×10^4 to 1×10^5 counts) in respect to *V. uliginosum* L.
 514 subsp. *gaultherioides* and *V. corymbosum* ones. Based on
 515 the [M]⁺ TOF accurate mass values determined for these
 516 analytes, the putative attribution to coumaroyl-hexoside
 517 anthocyanidin derivatives can be proposed ($\Delta \leq 1.9$ ppm),
 518 in agreement with the findings obtained under very similar
 519 chromatographic conditions by Zoratti and colleagues in

V. myrtillus fruits collected in the Alps of Northern Italy 520
 [35]. 521

Finally, peaks 61 (8.6×10^3 counts) and 64 (1.7×10^5
 522 counts), which were peculiar of *V. myrtillus* fruits, exhibited
 523 a single neutral loss of 338 Da (i.e., feruloyl-hexoside) with
 524 formation of the fragment at *m/z* 331.08 (i.e., malvidin).
 525 Accordingly, these peaks were tentatively identified as
 526 malvidin-feruloyl-hexosides. It is remarkable that these com-
 527 pounds, previously found in others fruits (e.g., grape) [36],
 528 were herein identified in bilberry for the first time. 529

530 *Flavonols*

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

540 Using the IDA TOF-Q/TOF workflow and comparisons
541 among retention times and mass spectra of unknown and au-
542 thentic standards, the unequivocal or at least tentative identi-
543 fication of 55 flavonols was achieved. As illustrated in
544 Table 2, among the identified flavonols, we found 36 glyco-
545 sides (peaks 66–71, 74–79, 82, 86–88, 91–94, 96–97, 99–101,
546 103–104, 107–110, 112–114, 116, and 117), 6 glucuronides
547 (peaks 65, 73, 83, 90, 106, and 111), 9 acyl derivatives (peaks
548 72, 80, 84, 85, 89, 95, 98, 102, and 105), and 4 aglycones
549 (peaks 81, 115, 118, and 119). TOF MS $[M-H]^-$ quasi-
550 molecular ions matched the proposed formulae with very high
551 mass accuracy, being Δ absolute values ≤ 2.5 ppm for 75% of
552 the identified analytes, and included in the range of 2.6–
553 4.4 ppm in the remaining cases (Table 2).

554 In agreement with literature findings [38], Q/TOF MS/MS
555 spectrum of flavonol glycosides exhibited both the heterolytic
556 and the homolytic cleavage of the glycosidic bond, producing
557 the aglycone fragment ion $[Y_0]^-$ and the radical aglycone ion
558 $[Y_0-H]^- \cdot$. Figure S5A of the ESM illustrates as an example
559 the MS/MS spectrum of quercetin-3-*O*-glucoside (peak 75), in
560 which the ions derived from heterolytic ($m/z = 301.03$) and
561 homolytic ($m/z = 300.03$) fission of the glycosidic bond are
562 shown. Neutral losses of 18 Da (H_2O), 28 Da (CO), and
563 30 Da (CH_2O), individual or combined one with the other,
564 have been also observed starting from the $[Y_0]^-$ ion (ESM
565 Fig. S5A), in agreement with characteristic MS/MS behavior
566 of flavonols elsewhere reported [37, 39]. Moreover, the loss of
567 15 Da ($-CH_3$) from the aglycone was occasionally observed
568 and putatively attributed to methoxylated flavonols (e.g., peak
569 78, tentatively identified as laricitrin-3-*O*-galactoside, see
570 Fig. S5B of the ESM). Further typical ions, at $m/z = 151.00$
571 and $m/z = 179.00$, originating from different retrocyclization
572 cleavages of the “C” ring and commonly identified as $^{1,3}A^-$
573 (retro-Diels-Alder) and $^{1,2}A^-$ fragments [40, 41], were ob-
574 served, even though with low signal intensity (ESM
575 Fig. S5A, B).

576 A different relative abundance of the aglycone fragment
577 $[Y_0]^-$ and the aglycone radical $[Y_0-H]^- \cdot$ ions, resulting from
578 heterolytic and homolytic cleavage of the glycosidic bond, has
579 been elsewhere demonstrated for kaempferol glycosides and
580 suggested also for other flavonol glycosides, on the basis of
581 the linkage position, as well as of the length of the saccharide

chain [38]. More in detail, if the sugar is a monosaccharide, 582
the cleavage of the 3-*O* position of the aglycone gives rise 583
preferentially to the $[Y_0-H]^- \cdot$ than the $[Y_0]^-$, as observed 584
for example in peaks 66–71 (Table 2). Conversely, in this 585
study, the preferential heterolytic cleavage of the monosaccha- 586
ride glycosidic bond was never observed, thus excluding the 587
presence of 7-*O*-glycoside derivatives of flavonols in the in- 588
vestigated *Vaccinium* species. Based on the aforementioned 589
considerations, peaks 66, 67, 78, 82, 86, 93, 97, 103, 107, 590
and 109 can be ascribed to 3-*O*-monohexoside flavonol deriva- 591
tives. More in detail, considering the whole mass dataset, as 592
well as the relative peak elution order, 3-*O*-galactoside and 3- 593
O-glucoside derivatives of myricetin (peaks 66 and 67), 594
laricitrin (peaks 78 and 82), kaempferol (peaks 86 and 93), 595
isorhamnetin (peaks 97 and 103), and syringetin (peaks 107 596
and 109) can be putatively identified, whereas peaks 71 and 597
75 were unequivocally identified as quercetin-3-*O*-galactoside 598
and quercetin-3-*O*-glucoside, due to the availability of the 599
authentic reference standards (Table 2). These flavonols were 600
found to be present in all the investigated *Vaccinium* species, 601
with the only exception of the glucoside derivative of 602
kaempferol in bilberry and blueberry, and of the glucoside 603
derivative of isorhamnetin in blueberry. It should also be 604
underlined that flavonol glucosides and especially galacto- 605
sides occurred with much higher signal intensity in 606
V. uliginosum L. subsp. *gaultherioides* berries, suggesting that 607
these analytes could be a typical metabolomic trait of “false 608
bilberry.” 609

610 Peaks 68–70, 77, 79, 87, 88, 91, 94, 99, 110, 112–114, 116,
611 and 117 showed the neutral loss of 132 Da, indicating the
612 presence of the aldopentose residue, and were attributed to
613 3-*O*-aldopentose derivatives of the aforementioned agly-
614 cones, on the basis of exact mass data of pseudo-molecular
615 ions and aglycone fragments. Interestingly, for the most abun-
616 dant aldopentose derivatives, a net predominance was ob-
617 served in “false bilberry,” whereas the others generally had
618 higher signal intensities in blueberry.

619 Peaks 92 and 104 were characterized by the neutral loss of
620 146 Da (i.e., deoxyhexose unit). The identity of the former
621 peak, which was found in both bilberry and blueberry, was
622 unequivocally attributed to quercetin-3-*O*-rhamnoside, using
623 the reference standard. Peak 104, which was found only in
624 blueberry, was identified as a laricitrin-deoxyhexoside and
625 putatively attributed to laricitrin-3-*O*-rhamnoside.

626 Data herein obtained for quercetin-3-*O*-rutinoside (peak
627 76, Fig. S6A of the ESM) and kaempferol-7-*O*-
628 neohesperidoside (peak 96, Fig. S6B of the ESM), which were
629 available as reference standards, suggested, also for flavonol
630 disaccharides, the higher abundance of homolytic or hetero-
631 lytic cleavages, as diagnostic of the 3-*O*- or 7-*O*-substitution,
632 respectively. However, according to Lu and coworkers [38], a
633 long saccharide chain substituted at the 3-*O* position, could
634 hinder the occurrence of the $[Y_0-H]^- \cdot$ 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 acetylhexosides 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 acetylhexose 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.

674 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 (Δ 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 gallicocatechin (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 \rightarrow C6 interflavanoid bond, instead of the C4 \rightarrow C8 one, is present between the two (E)C units.

739 The comparison between MS/MS spectra of B-type
740 procyanidins and peaks 120 and 122 highlighted m/z values
741 16 Da higher in most detected fragments of the latter peaks
742 (i.e., m/z 441.08, 423.07, and 305.06, see Fig. 1). Accordingly,
743 peaks 120 and 122 were tentatively identified as B-type
744 (E)GC-(E)GC dimers (Table 3). It is remarkable that for these
745 peaks, the fragment at m/z 177.02 has been also observed, in
746 contrast to MS/MS findings of (E)C dimers, in which this ion
747 was absent. It should also be noted that the fragment at m/z
748 177.02 was absent in (E)GC (see ESM Fig. S8A), thus sug-
749 gesting that it derives from the m/z 303.05 ion, as proposed in
750 Scheme 1.

751 Peak 170 showed the typical fragmentation of A-type
752 procyanidins (e.g., cleavage of the “C” ring, fission of the
753 heterogeneous C2 → O interflavanyl linkage and rearrange-
754 ment with formation of the ion at m/z 449.09), which was
755 unequivocally identified as procyanidin A2, based on its au-
756 thentic standard.

757 A number of proanthocyanidin trimers, tetramers, and
758 pentamers, characterized by B-type and both A- and B-type
759 (following A/B) interflavanyl linkages, were identified
760 (Table 3) on the basis of the typical mass fragmentation mech-
761 anisms of this polyphenol class: retro-Diels-Alder (RDA),
762 quinone methide formation (QM), and heterocyclic ring fis-
763 sions (HRF) [47].

764 Most of these proanthocyanidins were trimers (peaks 140,
765 144, 148, 157, and 164), tetramers (peaks 127, 138, 147, 151,
766 154, 155, 165, and 173), and pentamers (peaks 143, 146, 150,
767 158, 161, 166, and 167) consisting of only B-linked (E)C
768 units. More in detail, the MS/MS spectrum of B-type
769 procyanidin trimers was characterized by ions derived from
770 RDA fission (m/z 713.15) and successive loss of water (m/z
771 695.14), as well as the typical fragmentation pattern of B-type
772 procyanidin dimers (i.e., m/z 425.09 and 407.08) previously
773 discussed. Moreover, the presence of monomer (m/z 289.07
774 and 287.06) and dimer (E)C units (m/z 577.13 and 575.12)
775 derived from QM reaction confirmed the identity of B-type
776 procyanidin trimers. B-type procyanidin tetramers were de-
777 tected both as mono-charged (m/z 1153.26) and double-
778 charged (m/z 576.13) quasi-molecular ions and produced frag-
779 ments related to trimeric (m/z = 865.20 and 863.19), dimeric
780 (m/z = 577.13 and 575.12), and monomeric (m/z = 289.07 and
781 287.07) units, up to the characteristic MS/MS spectra of (E)C.
782 Similarly, B-type procyanidin pentamers showed the typical
783 fragmentations of the lower molecular weight B-type
784 procyanidin oligomers.

785 A/B-linked proanthocyanidin trimers (peaks 156, 168, and
786 171), tetramers (peaks 124 and 163), and pentamers (peaks
787 137 and 169) formed only of (E)C units were also putatively
788 identified. For these compounds, the RDA reaction affecting
789 the B-type-linked (E)C caused the ion at m/z 711.14 and, after
790 the loss of water, the ion at m/z = 693.13. The QM reaction
791 produced the fragments at m/z 573.10 and 289.07, whereas the

ions at 451.10 and 411.07 derived from HRF reactions. 792
Similarly, A/B-type procyanidin tetramers and pentamers 793
were identified on the basis of their MS/MS fragments 794
consisting of the abovementioned typical product ions of 795
A/B-type procyanidin trimer (m/z = 863.18) and dimer (m/z 796
 $=$ 575.12). 797

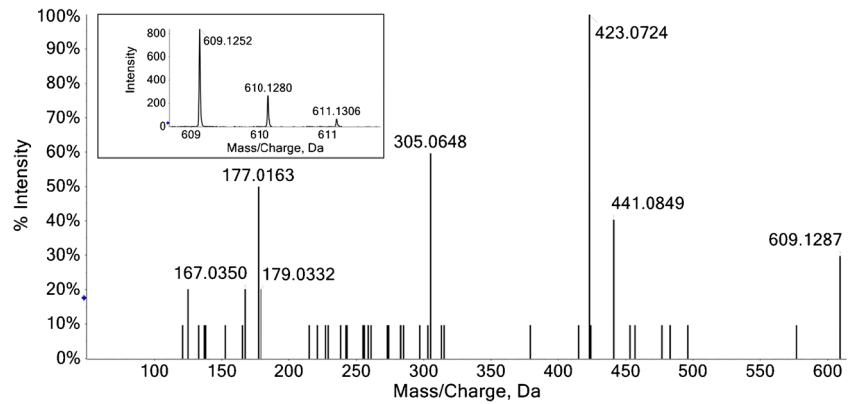
Interestingly, two compounds exclusively formed by 798
(E)GC units (i.e., peak 125, A/B-type trimer, and peak 128, 799
B-type trimer) were herein identified for the first time in 800
V. myrtillicus and *V. uliginosum* L. subsp. *gaultherioides* fruits. 801
Peak 125 was identified as A/B-type prodelphinidin trimer on 802
the basis of the characteristic fragments derived from RDA 803
(m/z = 743.13) and HRF (m/z 483.09 and 427.07) reactions, 804
whereas the MS/MS spectrum of peak 128 exhibited the prod- 805
uct ions corresponding to the formation of dimer (m/z 609.13) 806
and monomer (m/z 305.07) ions. In addition, the ion at m/z 807
541.08, derived from the cleavage of the “B” rings of the 808
trimer, was observed. 809

Four B-type proanthocyanidin trimers (peaks 129, 139, 810
149, and 160) and two tetramers (peaks 130 and 162) formed 811
by both (E)C and (E)GC monomers were also identified 812
(Table 3), but no information about the relative position of 813
the different units could be obtained by the MS/MS spectra. 814

Finally, six proanthocyanidin oligomers were identified as 815
trimers, tetramers, and pentamers consisting of both (E)C and 816
(E)GC units, linked with A/B-type (peaks 123, 131, 132, 134, 817
142, and 152) bonds. Interestingly, in this case, the fragmen- 818
tation spectra highlighted in most cases the diagnostic ions 819
that indicated the relative position of a certain monomer and/ 820
or the type of linkage (A type or B type) between two mono- 821
mers. For instance, peaks 142 and 152 were identified as A/B- 822
type trimers constituted by two units of (E)C and one unit of 823
(E)GC. For both peaks, the RDA reaction and the successive 824
loss of water, producing the ions at m/z 727.13 and 709.12, 825
were observed. Nevertheless, peak 142 was characterized by 826
fragments at m/z 427.07 and 451.10, fully consistent with the 827
occurrence in the molecule of (i) one terminal (E)GC linked to 828
one (E)C by an A-type linkage and (ii) two (E)C units linked 829
by a B-type interflavanyl bond, respectively. Moreover, a 830
high-intensity fragment at m/z 289.07 was observed, in accord- 831
ance with the presence of a B-type terminal (E)C (Fig. 2A). 832
Analogously, the MS/MS spectrum of peak 152 showed frag- 833
ments at m/z 411.07 and 467.10, which are in agreement with 834
the presence of (i) one terminal (E)C linked to the other por- 835
tion of the molecule by an A-type linkage and (ii) one (E)C 836
and one (E)GC unit linked each other by a B-type bond. 837
Moreover, it should be noted that an intense fragment at m/z 838
305.06, attributable to the (E)GC unit, was also observed 839
(Fig. 2B). Accordingly, peaks 142 and 152 were putatively 840
identified as A/B-type (E)GC-(E)C-(E)C and A/B-type 841
(E)C-(E)C-(E)GC, respectively. 842

A similar consideration can be done for peaks 132 and 134, 843
which were A/B-type trimer constituted by one unit of (E)C and 844

Fig. 1 Q/TOF MS/MS spectrum of peaks 120 and 122, identified as B-type dimer of (epi)gallocatechin

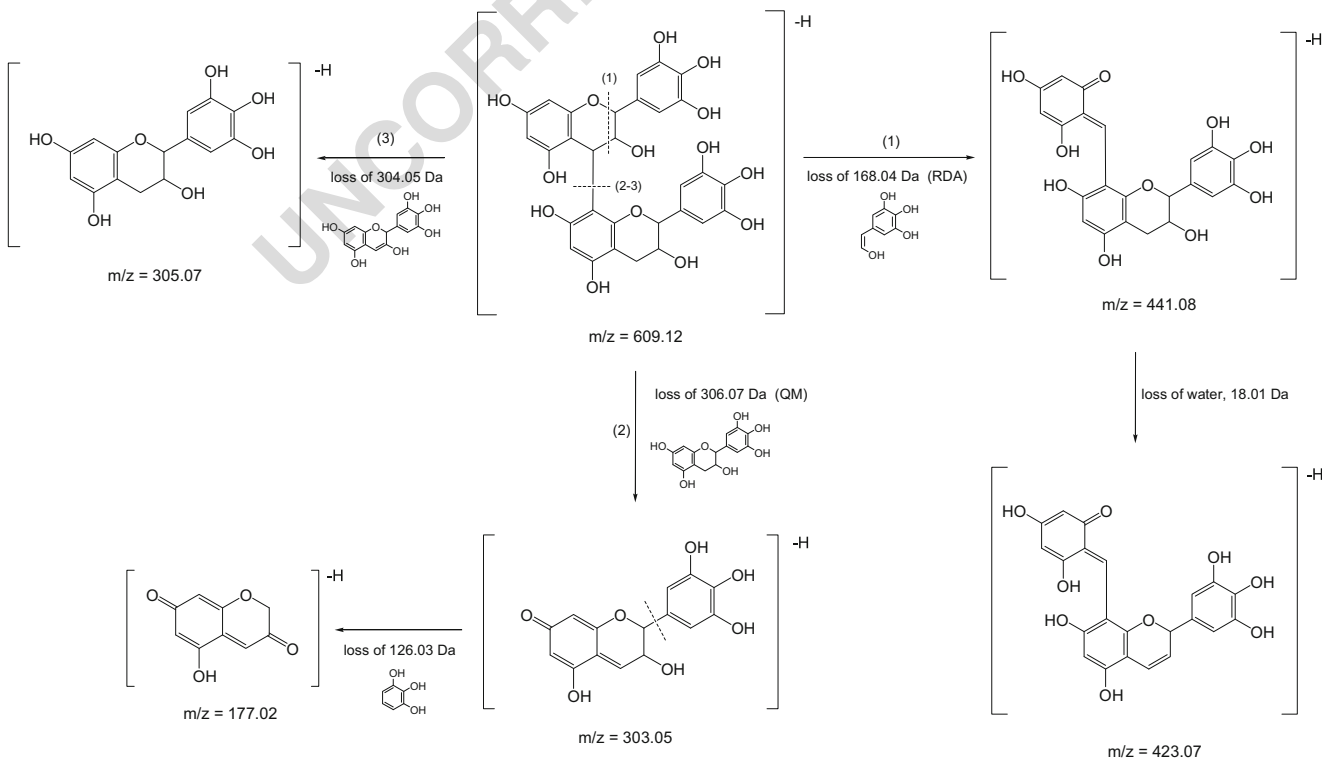


845 two units of (E)GC. In both these peaks, the ion at m/z 427.07
 846 indicated the presence of one terminal (E)GC unit linked with an
 847 A-type bond with the rest of the molecule, whereas the ion at m/z
 848 467.10 was diagnostic for the occurrence of B-type-linked
 849 (E)GC and (E)C. However, 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

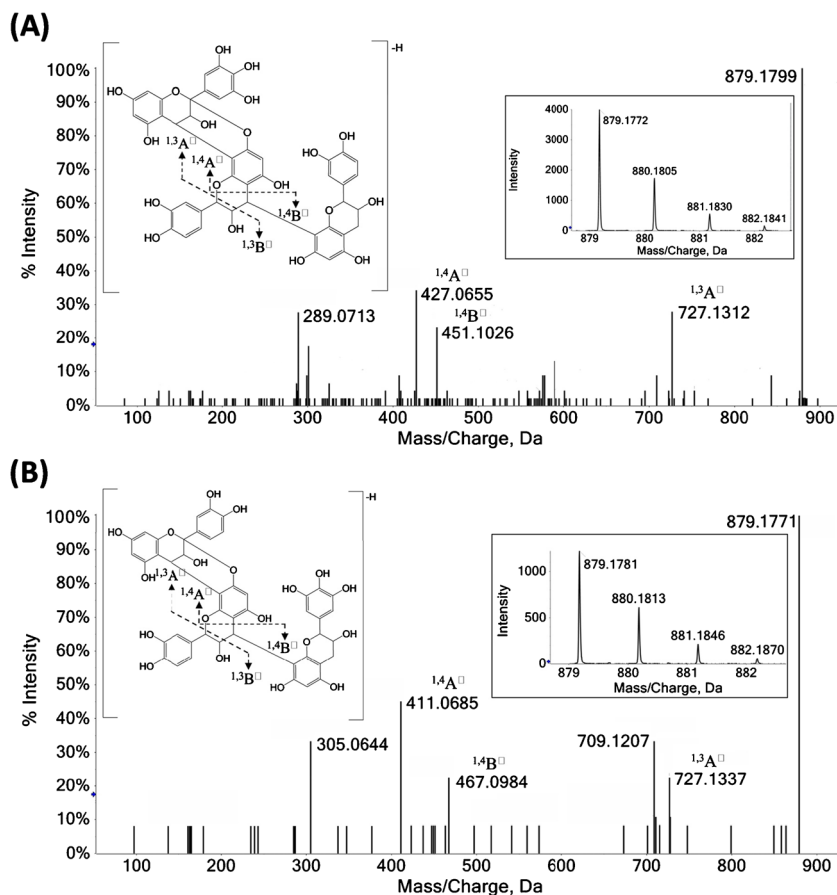
857 fragmented originating the ion at m/z 863.18, which is consis-
 858 tent with the formation of an (epi)catechin trimer with one A-
 859 type and one B-type linkage, together with other characteristic
 860 ions (i.e., m/z 711.14, 573.10, and 411.07), deriving from the
 861 catechin trimer fragmentation. 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



869 *Other compounds*

870 Using the IDA TOF-Q/TOF workflow under negative ioniza- 870
 871 tion, 29 further phenolic compounds belonging to various 871
 872 classes were putatively or unequivocally identified in berry 872
 873 samples (Table 4). Also in these cases, a very good agreement 873
 874 between TOF MS quasi-molecular ions and proposed formulae 874
 875 was obtained (Δ absolute values ≤ 3.8 ppm). 875

876 Peak 199 was found to be common to the three species and 876
 877 unequivocally identified as phloridzin after comparison with 877
 878 the corresponding authentic standard. The use of the reference 878
 879 standard allowed for certainly identifying also peak 190 as 879
 880 scopoletin, which was detected only in “false bilberry.” 880

881 Peak 174, which was present at quite similar intensities in 881
 882 all berry species, was unambiguously identified as gallic acid, 882
 883 due to the availability of the authentic reference standard. 883

884 Several phenolic acids belonging to the class of the 884
 885 hydroxycinnamic acids (peaks 175–178, 180, 182, 185, 186, 885
 886 192, 193, 197, and 198) were also putatively or unequivocally 886
 887 identified, depending on the availability of the authentic stan- 887
 888 dards. These compounds were generally found at higher intensity 888
 889 in *V. corymbosum* and in some cases (peaks 176, 177, 889
 890 193, 197, and 198) detected exclusively in this berry species. 890

891 Peak 179 exhibited a quasi-molecular ion at m/z 353.08, 891
 892 which fragmented giving rise to a neutral loss of 162 Da and 892

893 ions attributable to caffeic acid. This peak was therefore puta- 893
 894 tively attributed to a caffeic acid hexoside. Analogously, peaks 894
 895 181 and 183 were tentatively identified as coumaric acid and 895
 896 ferulic acid hexosides. 896

897 Peaks 188, 194, and 202 showed pseudo-molecular ions at 897
 898 m/z 335.08, 367.10, and 445.11, respectively, and shared the 898
 899 typical fragments of caffeic acid (Table 4), thus suggesting 899
 900 their putative attribution as caffeic acid derivatives. 900

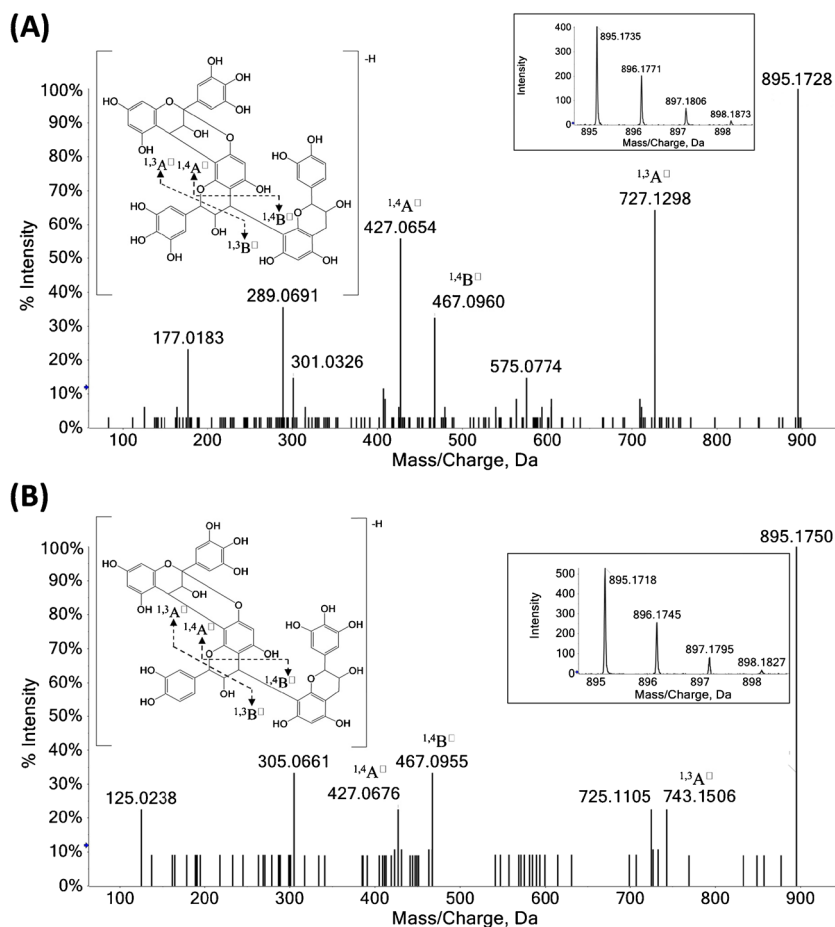
901 For peaks 201 and 202, the same quasi-molecular ion at m/z 901
 902 411.17 was found. The fragmentation gave rise to a neutral 902
 903 loss that corresponded to a malonyl hexoside (248 Da) with 903
 904 formation of a fragment consistent with coumaric acid [M - 904
 905 H] $^-$ ion (m/z 163.04), as well as various fragments typical 905
 906 of coumaric acid, thus suggesting the putative identification of 906
 907 both peaks as coumaric acid-malonyl-hexosides. 907

908 Fragmentations of the quasi-molecular [$M-H$] $^-$ ions of 908
 909 peaks 195 and 196 (m/z 535.15) were in full agreement with 909
 910 data reported by Hokkanen et al. [44] for coumaroyl iridoids. 910
 911 These peaks, previously identified in *V. myrtillus* fruits [48], 911
 912 were much more intense in bilberry than in “false bilberry,” 912
 913 whereas these were absent in blueberry. 913

914 Peaks 184, 187, 189, and 191 were putatively identified as 914
 915 cinchonain II isomers, in agreement with the fragmentation 915
 916 scheme reported by Hokkanen and coworkers [44]; all these 916
 917 compounds have been found in *V. corymbosum* berries, 917

Study and evaluation of the polyphenolic composition of *Vaccinium*

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



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

923 **Comparison of polyphenolic compositions by PCA**

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

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

hexosides (peaks 61 and 64) and various anthocyanidin glyco-
 sides, such as dihexosides (e.g., peaks 3, 5, and 13) and
 aldopentose-hexosides (e.g., peaks 21 and 27). An opposite be-
 havior (i.e., strong and negative correlation with PC1) was ob-
 served 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 (ex-
 plained 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).

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

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

976 PCA was also applied to the TOF data acquired in negative
 977 mode, highlighting two factors with eigenvalues higher than 1,
 978 which accounted for a total explained variance of 96.4% (66.3%
 979 and 30.1% for PC1 and PC2, respectively). The variable separa-
 980 tion on the two PCs was in this case not as good as that obtained
 981 for compounds detected under positive ionization, probably also
 982 due to the much higher number of analytes detected in negative
 983 polarity. In fact, many metabolites were distributed in a very wide
 984 range of negative PC1 values, with both positive and negative
 985 loadings on PC2. However, some analytes showed very high and
 986 positive loadings on PC1 and a very narrow range of PC2 values,
 987 thus forming a cluster (see Fig. 5A and the zoomed area). This
 988 cluster contained all the identified flavonol acetyl-hexosides
 989 (e.g., peaks 89 and 105), some flavonol aldopentosides (e.g.,
 990 peaks 69, 77, and 87), and a number of B-type proanthocyanidins
 991 eluting at relatively low retention times, whereas no A/B-type
 992 derivatives were found in this group. Furthermore, some
 993 hydroxycinnamic acids, such as neochlorogenic (peak 175),
 994 cryptochlorogenic (peak 182), and coumaroylquinic (peak
 995 177), belonged to the cluster. Cinchonains exhibited high load-
 996 ings on PC1 and low loadings on PC2, as well. Conversely, very
 997 high and negative values on the former latent variable were ob-
 998 served for all flavonol glucuronides (e.g., peaks 65, 73, 83, 90,
 999 106, and 111).

1000 The score plot (Fig. 5B) highlighted a very good accuracy and
 1001 precision of PCA also for data obtained under negative ioniza-
 1002 tion, with quality control samples well centered on the origin of
 1003 the PC coordinates and evident separations among
 1004 *V. corymbosum* (high scores on PC1 and close to zero on PC2),
 1005 *V. myrtillus* (high and negative scores on PC1 and very high and
 1006 positive on PC2), and *V. uliginosum* subsp. *gaultherioides* (neg-
 1007 ative scores on both PC1 and PC2) berries. Thus, LC-ESI-MS/
 1008 MS in negative ionization gave useful and complementary infor-
 1009 mation with respect to the positive mode for the discrimination of
 1010 the investigated species.

1011 **Conclusions**

1012 LC-ESI-TOF and LC-ESI-Q/TOF analysis, performed both in
 1013 positive and negative modes, allowed to obtain a

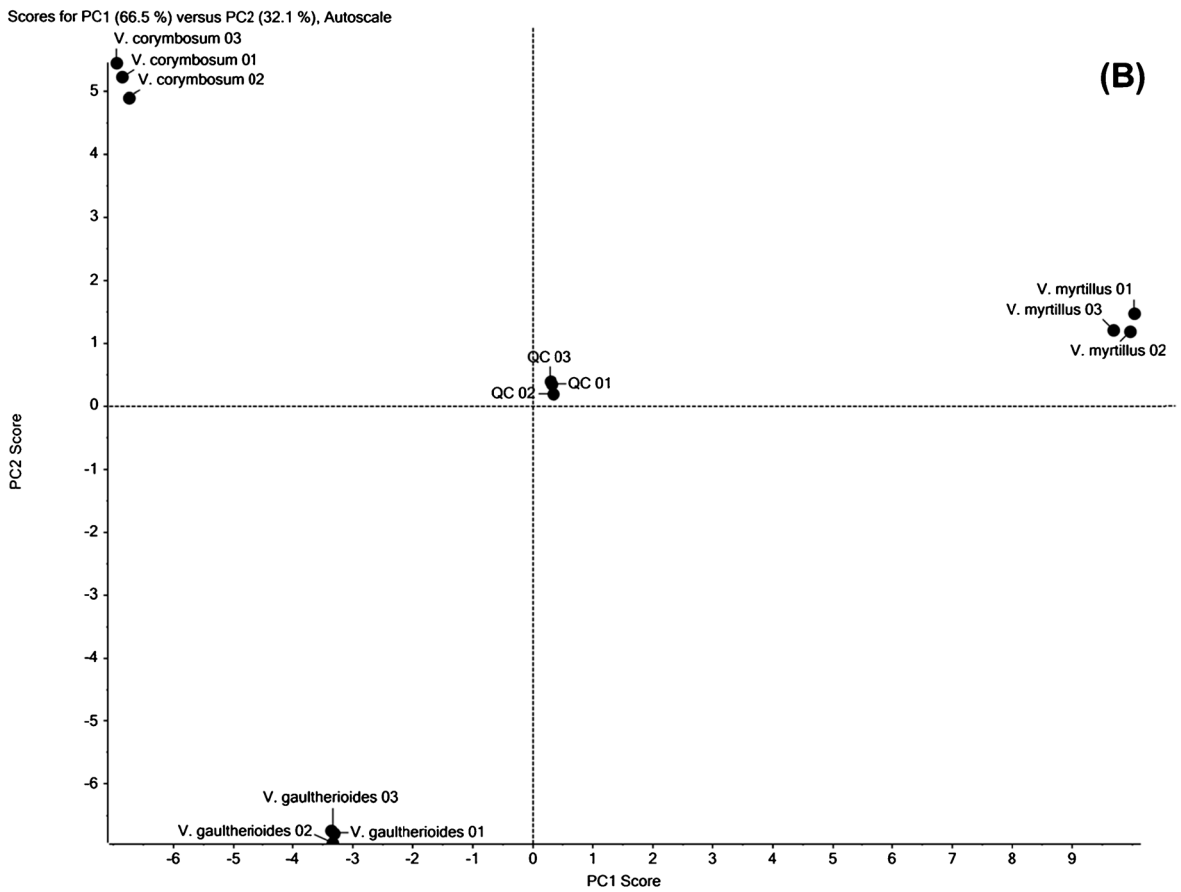
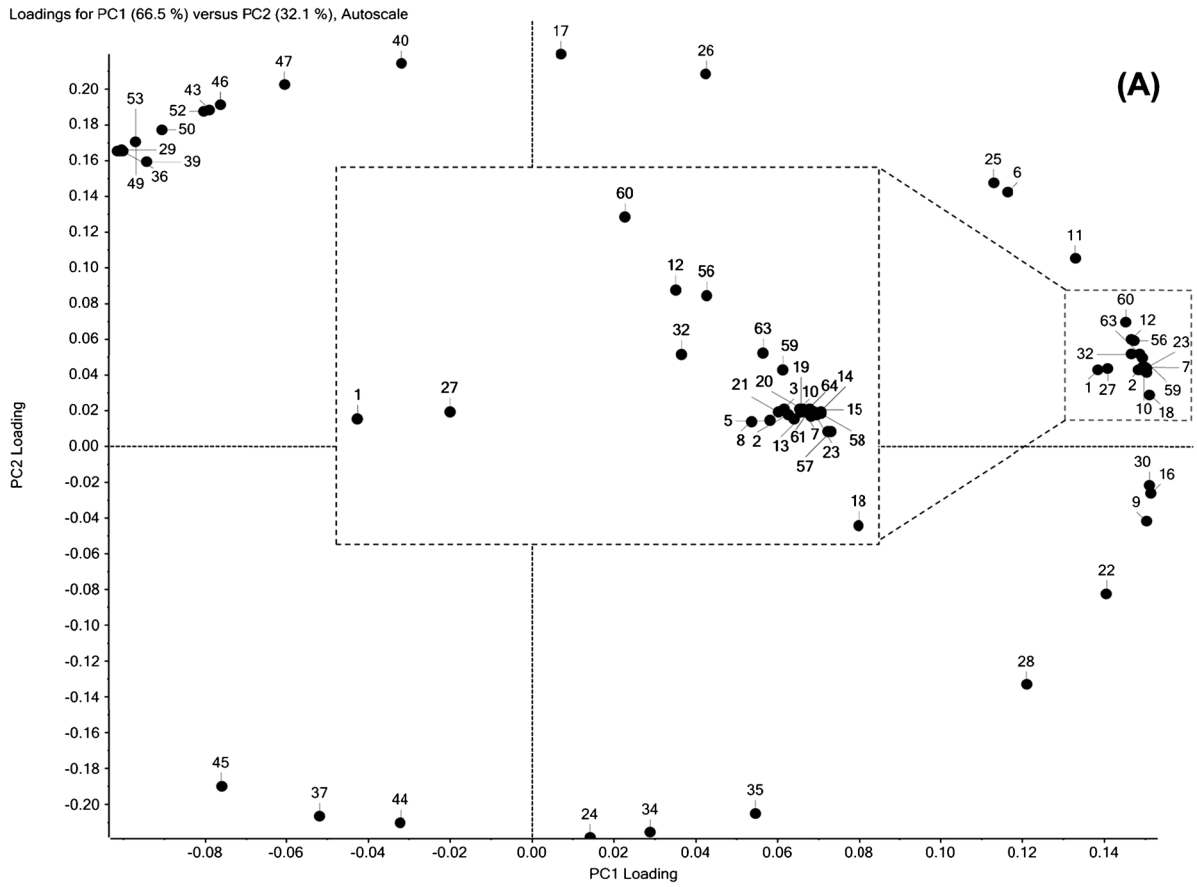
comprehensive picture of the polyphenolic composition of
V. myrtillus, *V. corymbosum*, and *V. uliginosum* subsp.
gaultherioides berries.

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 re-
 ported 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.

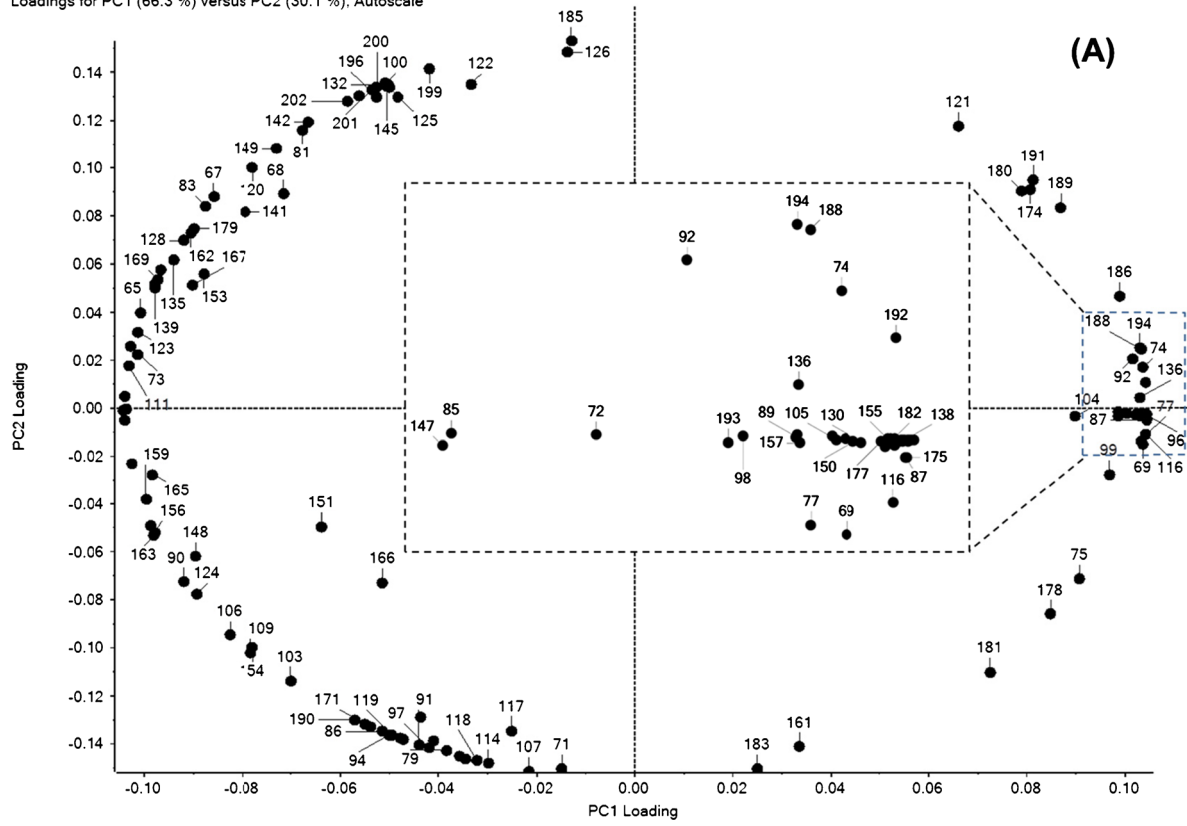
Flavonols resulted generally more abundant in
V. corymbosum. In fact, 51 of the 55 flavonols identified here-
 in were found to be present in blueberries whereas only 37 in
 “false bilberry” and 35 in bilberry. Remarkably, in previous
 works, the flavonol derivatives discussed above were only
 partially 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 flavo-
 nol glycosides a more comprehensive study of such metabo-
 lites 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, in-
 cluding trimers, tetramers, and pentamers, were found to be
 present in all species. However, some species-specific metabo-
 lites 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 com-
 pounds eluted at earlier retention times, compared to the me-
 tabolites common to the three species, thus suggesting a great-
 er presence of catechin and gallocatechin, rather than the cor-
 responding 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

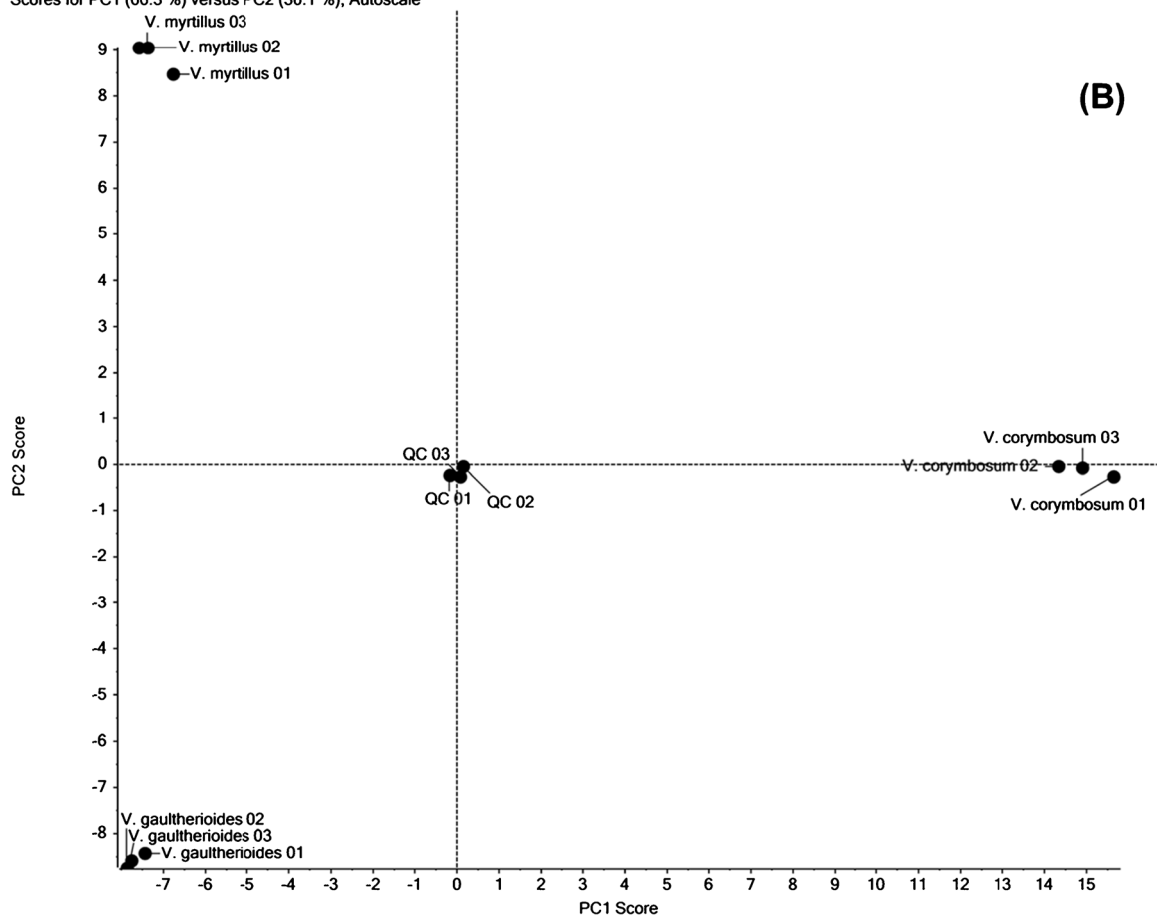
Fig. 4 Loading (A) and score (B) plots of PC1 versus PC2 (PCA of
 original LC-ESI-TOF MS data acquired in positive ionization).
 Numbers shown in the loading plot refer to the peak numbers reported
 in Table 1



Loadings for PC1 (66.3%) versus PC2 (30.1%), Autoscale



Scores for PC1 (66.3%) versus PC2 (30.1%), Autoscale



◀ **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

1062 reported in literature [13, 44], this study provides a much more
1063 detailed description of the flavanol composition in these
1064 berries, identifying for the first time a large number of
1065 proanthocyanidins with high molecular weight.

1066 All the aforementioned LC-MS data were well-integrated
1067 using the PCA approach, which demonstrated to be suitable
1068 for a clear discrimination of the investigated berry species
1069 both in positive and negative ionization modes.

1070 The comprehensive investigation herein illustrated, which
1071 evidenced phenolic metabolites exclusively detected in one
1072 species or characterized by extremely different intensities in
1073 the three berries, can be useful for future developments of
1074 methods aiming at evaluating the quality of *Vaccinium* berry
1075 transformation products and to avoid frauds. These products,
1076 in fact, are not only fruit juices or jams that are not subjected to
1077 any particular regulation concerning their phenolic content but
1078 also supplements or actual drugs, which must conversely re-
1079 spect what is written in the label, both in terms of plant material
1080 used for its preparation and amount of active ingredients
1081 contained in the product.

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1087 **Compliance with ethical standards**

1088 **Conflict of interest** The authors declare that they have no conflict of
1089 interest.

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