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# Small berries with big nutritional benefits

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*Berries are amongst the richest sources of phenolic compounds in the human diet. Some of the phenolic compounds found in berries are present in many other fruits and vegetables (e.g. anthocyanins), while others (e.g. ellagitannins) are specific to some species of berries. Anthocyanins are widespread in the human diet, being responsible for the color of red berries, red grape and wine, and many vegetables. In order to give a complete picture of their presence in the human diet, the anthocyanins were quantified at molecular level by means of HPLC-DAD and HPLC-DAD-ESI-MS techniques in 282 samples of soft fruits and strawberry grown in Trentino. The average content of anthocyanins was the highest in blueberry (1962 mg/kg), followed in decreasing order by black currant (1279 mg/kg), blackberry (1022 mg/kg), strawberry (367 mg/kg), raspberry (313 mg/kg), wild strawberry (198 mg/kg), gooseberry (78 mg/kg) and red currant (69 mg/kg). The variability inside each species is extremely high and a careful choice of the variety is critical for assuring an optimal presence of anthocyanins in the fruit.*
*The ellagitannins of 21 raspberry and 15 blackberry cultivars produced in Trentino were extracted and characterized. This study describes the application of a new procedure for acid hydrolysis of* Rubus *ellagitannins in methanol, which allows quantification of all the major reaction products ellagic acid, methyl-sanguisorboate, methylgallate and sanguisorbic acid. The total amount of ellagitannins varied from 941.5 mg/kg to 1742.7 mg/kg for raspberry samples and from 880.2 mg/kg to 3897.5 mg/kg for blackberry samples. The total content of ellagitannins was found to vary by a factor of 2 among raspberry cultivars and by a factor of 4 among blackberry cultivars.*
**Keywords:** small berries, polyphenols, anthocyanins, ellagitannins

***Kleine Beeren mit großem Nährwert.*** *Beeren gehören zu den besten Quellen phenolischer Verbindungen in der menschlichen Ernährung. Einige der Phenolverbindungen von Beeren finden sich in vielen anderen Früchten und Gemüsen (z. B. Anthocyane), während andere (z. B. Ellagitannine) spezifisch für einige Arten von Beeren sind. Anthocyane sind in der menschlichen Ernährung häufig zu finden, sie sind verantwortlich für die rote Farbe von Beeren, Trauben, Wein und vielen Gemüsen. Um ein vollständiges Bild ihres Vorkommens in Nahrungsmitteln zu geben, wurden die Anthocyane auf molekularer Ebene mittels HPLC-DAD und HPLC-DAD-ESI-MS-Techniken in 282 Proben von Beerenobst und Erdbeeren aus dem Trentino quantifiziert. Der durchschnittliche Gehalt an Anthocyanen war am höchsten in Heidelbeeren (1962 mg/kg), gefolgt von Schwarzen Johannisbeeren (1279 mg/kg), Brombeeren (1022 mg/kg), Erdbeeren (367 mg/kg), Himbeeren (313 mg/kg), Walderdbeeren (198 mg/kg), Stachelbeeren (78 mg/kg) und Roten Johannisbeeren (69 mg/kg). Die Schwankungen innerhalb der einzelnen Arten sind sehr hoch, und eine sorgfältige Sortenwahl ist entscheidend, um optimale Gehalte an Anthocyanen in der Frucht zu sichern. Die Ellagitannine von 21 Himbeer- und 15 Brombeersorten aus dem Trentino wurden extrahiert und charakterisiert. Diese Studie beschreibt die Anwendung eines neuen Verfahrens für die saure Hydrolyse der Ellagitannine von Rubus-Arten in Methanol, das die Quantifizierung aller wichtigen Reaktionsprodukte (Ellagsäure, Methylsanguisorboat, Methylgallat und Sanguisorbinsäure) erlaubt. Der Gesamtgehalt der Ellagitannine variierte von 941,5 mg/kg bis 1742,7 mg/kg bei Himbeerproben (Faktor 2) und von 880,2 mg/kg bis 3897,5 mg/kg bei Brombeerproben (Faktor 4).*
**Schlagwörter:** Beerenobst, Polyphenole, Anthocyan, Ellagitannin

There is consistent epidemiological evidence linking consumption of a diet rich in fruit and vegetables with a reduced incidence of cancer, coronary disease and neurological disorders. The protective effects of dietary fruit and vegetables are attributed to their relatively high content of antioxidants, of which polyphenols are quantitatively the most significant (KALT et al., 1999; ANCOS et al., 2000; KÄHKÖNEN et al., 2001). Berries are amongst the richest sources of phenolic compounds in the human diet (KÄHKÖNEN et al., 2001). Some of the phenolic compounds found in berries are present in many other fruits and vegetables (e.g. anthocyanins), while others (e.g. ellagitannins) are specific to some species of berries.

Anthocyanins are widespread in the human diet, being responsible for the color of red berries, red grape and wine and many vegetables. Due to their complex, hydrophilic and bulky structure, anthocyanins are among the least absorbed phenols (MANACH et al., 2005). It was demonstrated, that they can reach as intact molecules the mammalian plasma (PASSAMONTI et al., 2003), liver (PASSAMONTI et al., 2005a) and brain (PASSAMONTI et al., 2005b) within minutes from their introduction into the stomach. Information on structures and concentrations of anthocyanins is still incomplete. Different anthocyanins may have significantly different chemical and physiological properties, therefore it is critical to know the distribution and chemical structures of anthocyanins in foods (WU and PRIOR, 2005).

In red raspberries and blackberries ellagitannins are the main class of phenolic compounds and, therefore this type of fruit represents one of the richest sources of ellagitannins in the human diet. The metabolism of ellagitannins in humans has been extensively investigated, and some of the pharmacological properties of their biomarkers, urolithins, have recently been characterised (CERDA et al., 2005; LARROSA et al., 2006).

Lambertianin C and sanguiin H-6 were shown to be the major ellagitannins of *Rubus* berries, lambertianin C being the major ellagitannin in blackberries and sanguiin H-6 in raspberries. Beside these two compounds, another 20 minor ellagitannins were detected and preliminarily characterized in *Rubus* extracts, together with ellagic acid and four different ellagic acid conjugates (GASPEROTTI et al., 2010).

In view of the increasing consumer attention to the nutritional value of their diets, the antioxidant content of berries and in particular their phenolic profile ought to be considered as an important trait for breeding programs. This implies a shift from traditional selection criteria focused on yield, pest resistance and shelf-life and the establishment of screening programs that establish genotypic differences in species in relation to berry phenolics composition.

## Materials and methods

### Anthocyanins

**Samples.** 47 samples of strawberries, 23 samples of wild strawberries, 29 samples of red currants, 103 samples of raspberries, 15 samples of blackberries, 39 samples of blueberries, 8 samples of gooseberries, 18 samples of black currants grown in Trentino were included in the study.

**Sample extraction**. Polyphenols were extracted following the method of MATTIVI et al. (2002). Before extraction, the fruit and extraction solution were cooled to 4 °C to limit enzymatic and chemical reactions. 60 g of fresh fruit were homogenised in a blender (Osterizer model 847-86) at speed one, in two times 100 ml of mixture acetone/water (70/30 v/v) for 1 min and made up to 250 ml with the same solvent. The centrifuged extracts were stored at -20 °C until analysis.

**Sample preparation**. To remove acetone from the extract, 20 ml of extract was evaporated to about one third of the volume in a 100 ml pear-shaped flask, by rotary evaporation and under reduced pressure, at 35 °C. The sample was brought back to 20 ml with deionized water and filtered through 0.45 µm, 13 mm PTFE

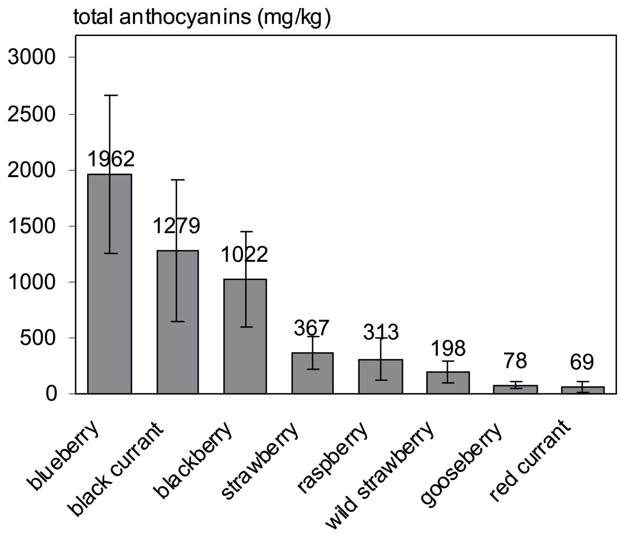


Fig. 1: The amounts of total anthocyanins in soft fruits and strawberry

syringe-tip filters (Millipore, Bedford, MA) into LC vials for HPLC analysis.

**Methods**. Analyses of anthocyanins were carried out on a HPLC system (Waters 2695 equipped with Waters 2996 DAD, Milford, MA). Separation was performed using a C18 RP column (Xterra MS C18, 3.5 µm, 2.1 x 150 mm, Waters Corp., Milford, MA) proteced by a corresponding precolumn (Xterrra MS C18, 3.5 µm, 2.1 x 10 mm, Waters Corp., Milford, MA). The mobile phases for the HPLC analysis of anthocyanins consisted of 5 % formic acid in water (A) and 5 % formic acid in methanol (B). The column was equilibrated for 7 min prior to each analysis. The flow rate was 0.2 ml/min. The UV-VIS spectra were recorded from 230 to 600 nm, with detection at 520 nm. Because of the coelution of some anthocyanins in blueberry samples peaks were quantified by electrospray ionisation-mass spectrometer (ESI-MS) (model ZQ, Micromass, Manchester, UK).

## Ellagitannins

**Plant material**. Plants of 21 raspberry and 15 blackberry samples of different cultivars grown in Trentino, Italy were included in the study.

Sample preparation and the analytical method are described in Vrhovsek et al. (2008).

# Results and discussion

## Anthocyanins

In order to give a complete picture of their presence in the human diet, the anthocyanins were quantified

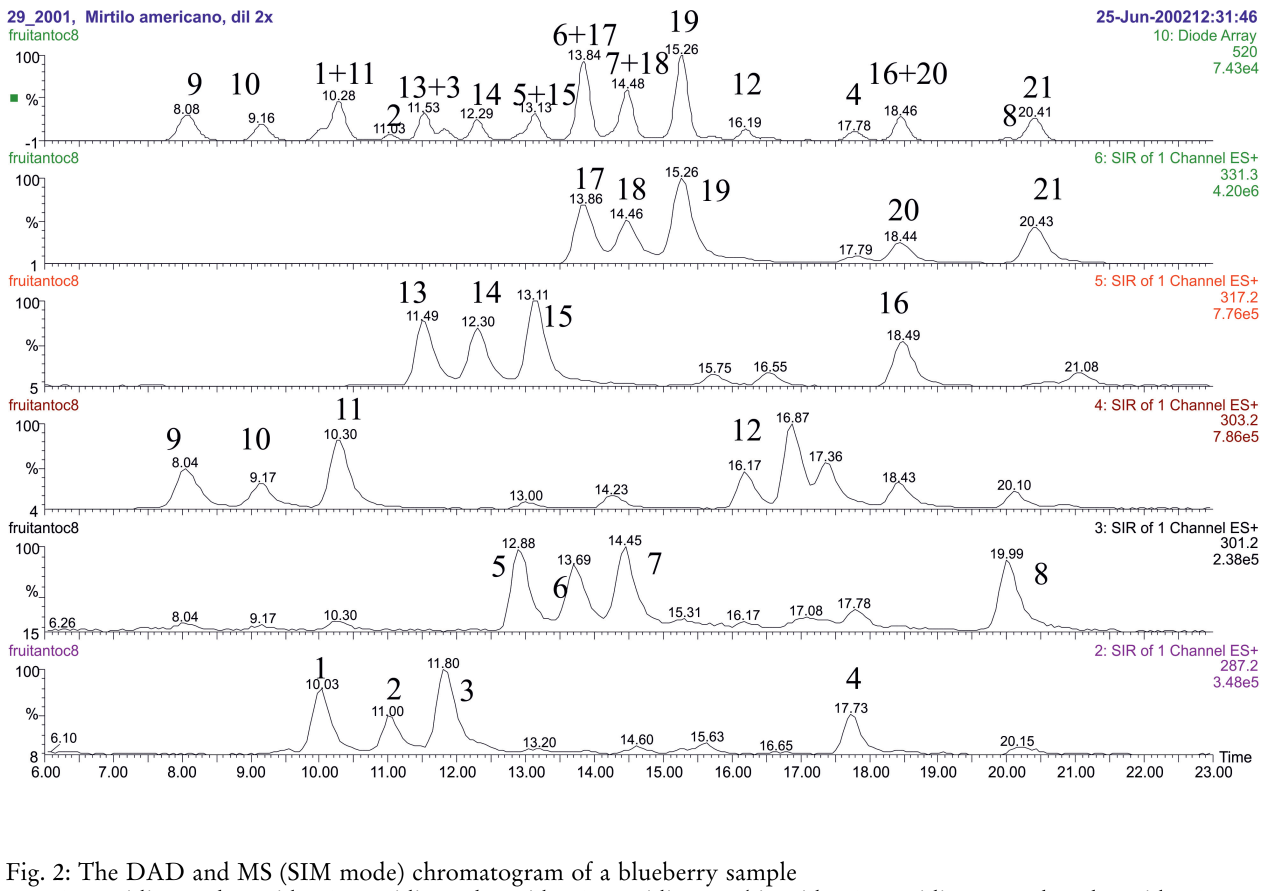


Fig. 2: The DAD and MS (SIM mode) chromatogram of a blueberry sample

1) cyanidin 3-galactoside; 2) cyanidin 3-glucoside, 3) cyanidin 3-arabinoside, 4) cyanidin 6-acetyl-3-glucoside, 5) peonidin 3-galactoside, 6) peonidin 3-glucoside, 7) peonidin 3-arabinoside, 8) peonidin 6-acetyl-3-glucoside, 9) delphinidin 3-galactoside, 10) delphinidin 3-glucoside, 11) delphinidin 3-arabinoside, 12) delphinidin 6-acetyl-3-glucoside, 13) petunidin 3-galactoside, 14) petunidin 3-glucoside, 15) petunidin 3-arabinoside, 16) petunidin 6-acetyl-3-glucoside, 17) malvidin 3-galactoside, 18) malvidin 3-glucoside, 19) malvidin 3-arabinoside, 20) malvidin 6-acetyl-3-galactoside, 21) malvidin 6-acetyl-3-glucoside

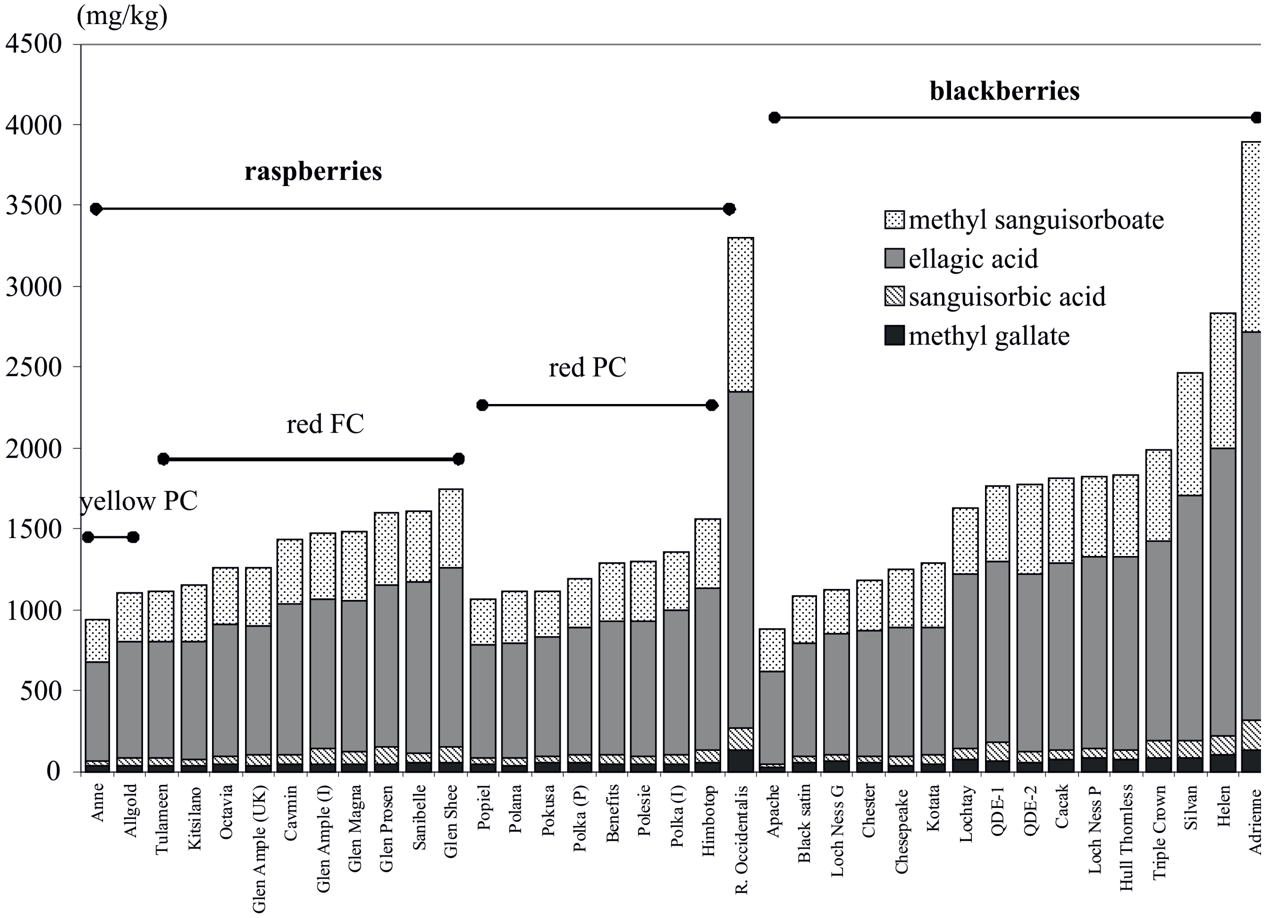


Fig. 3: The content of ellagitannins in different raspberry and blackberry varieties

at the molecular level in 282 samples of soft fruits and strawberries grown in Trentino. The concentrations of anthocyanins differ a lot in different fruits (Fig. 1). The concentrations differ significantly also between the varieties of single fruit (Fig. 1). Blueberries contain a very complex mixture (up to 23 compounds) of simple monosaccharides of delphinidin, petunidin, malvidin, cyanidin, peonidin and acylated pigments (Fig. 2). Strawberry and wild strawberry contain five and six main pigments, respectively, containing mostly pelargonidin 3-glucoside with lower quantities of other derivatives of pelargonidin, including their malonic esters, and cyanidin derivatives. Raspberry, red currant and blackberry contain a mixture of seven, six and six pigments, respectively, mainly complex glycosides of the cyanidin, qualitatively similar for raspberry and red currant, including pigments acylated with malonic acid and dioxalic acid in the blackberry. Black currant contains eight main pigments, mainly delphinidin and cyanidin 3-rutinoside, with lower amounts of other glycosides and of pigments acylated with p-coumaric acid. The gooseberry has a pattern of eight main pigments, mainly derivatives of cyanidin and peonidin, including their 3-glucoside, 3-rutinoside, 3-galactoside and both p-coumaric and caffeic acid acylated anthocyanins.

The average content of anthocyanins was the highest in blueberry (1962 mg/kg), followed in decreasing order by black currant (1279 mg/kg), blackberry (1022 mg/kg), strawberry (367 mg/kg), raspberry (313 mg/kg), wild strawberry (198 mg/kg), gooseberry (78 mg/kg) and red currant (69 mg/kg). The variability within each species is extremely high and a careful choice of the variety is critical for assuring an optimal presence of anthocyanins in the fruit.

## Ellagitannins

The study provided an insight into the variability of the ellagitannins in 37 important *Rubus* genotypes. The products of ellagitannins acid hydrolysis - ellagic

Table1: Identification of anthocyanins from different berries using HPLC-DAD-ESI/MS

| COMPOUND | AGLYCON | SUGAR | Rt (min) | max (nm) | MOL. ION (m/z) | BASE PEAK (m/z) | Highbush Blueberry | Strawberry | Raspberry | Red currant | Blackberry | Black currant |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cyanidin 3,5-diglucoside | cyanidin | glucose | 7,45 | 515 | 647 | 287,3 | | | X | | | |
| cyanidin 3-coumaroylglucoside | cyanidin | glucose | 22,38 | 520 | 595 | 287,3 | | | | | | X |
| cyanidin 3-dioxalylglucoside | cyanidin | glucose | 16,41 | 516 | 593 | 287,3 | | | | | X | |
| cyanidin 3-rutinoside-5-glucoside | cyanidin | glucose | 10,59 | 515 | 757 | 287,3 | | | X | X | | |
| cyanidin 3-xylosil-rutinoside | cyanidin | xylose+rutinose | 11,81 | 516 | 727 | 287,3 | | | X | X | | |
| cyanidin 3-arabinoside | cyanidin | arabinose | 11,86 | 516 | 419 | 287,3 | X | | | | X | |
| cyanidin 3-galactoside | cyanidin | galactose | 10,08 | 515 | 449 | 287,3 | X | | | | | |
| cyanidin 3-glucoside | cyanidin | glucose | 11,10 | 515 | 449 | 287,3 | X | | X | | X | |
| cyanidin 3-glucoside-malonat | cyanidin | glucose | 15,30 | 510 | 535 | 287,2 | | | | | X | |
| cyanidin 3-rutinoside | cyanidin | rutinose | 12,07 | 518 | 595 | 287,3 | | | X | X | X | X |
| cyanidin 3-sophoroside | cyanidin | sophorose | 9,97 | 516 | 611 | 287,2 | | | X | X | | |
| cyanidin 6-acetyl-3-glucoside) | cyanidin | glucose | 17,73 | 526 | 491 | 287,3 | X | | | | | |
| cyanidin 3-sambubioside | cyanidin | glucose+xylose | 11,24 | 516 | 581 | 287,3 | | | X | | | |
| cyanidin 3-xyloside | cyanidin | xylose | 14,80 | 516 | 419 | 287,2 | | | | | X | |
| delphinidin 3-coumaroylglucoside | delphinidin | glucose | 20,88 | 527 | 611 | 303,2 | | | | | | X |
| delphinidin 3-arabinoside | delphinidin | arabinose | 10,36 | 524 | 435 | 303,3 | X | | | | | |
| delphinidin 3-galactoside | delphinidin | galactose | 8,20 | 523 | 465 | 303,3 | X | | | | | |
| delphinidin 3-glucoside | delphinidin | glucose | 9,23 | 523 | 456 | 303,3 | X | | | | | X |
| delphinidin 3-rutinoside | delphynidin | rutinose | 10,50 | 526 | 611 | 303,2 | | | | | | X |
| delphinidin 6-acetyl-3-glucoside | delphinidin | glucose | 16,17 | 529 | 507 | 303,3 | X | | | | | |
| malvidin 3-arabinoside | malvidin | arabinose | 15,26 | 527 | 463 | 331,3 | X | | | | | |
| malvidin 3-galactoside | malvidin | galactose | 13,86 | not pure | 493 | 331,3 | X | | | | | |
| malvidin 3-glucoside | malvidin | glucose | 14,51 | 527 | 493 | 331,3 | X | | | | | |
| malvidin 6-acetyl-3-galactoside | malvidin | galactose | 18,40 | not pure | 535 | 331,3 | X | | | | | |
| malvidin 6-acetyl-3-glucoside | malvidin | glucose | 20,43 | 528 | 535 | 331,2 | X | | | | | |
| 5-carboxypyranopelargonidin 3-glucoside | pelargonidin | glucose | 15,32 | 503 | 501 | 271,2 | | X | | | | |
| pelargonidin 3-acetylglucoside | pelargonidin | glucose | 19,69 | 503 | 475 | 271,2 | | X | | | | |
| pelargonidin 3-sambubioside | pelargonidin | glucose+xylose | 13,03 | 503 | 565 | 271,2 | | | X | | | |

Table1 (Continue): Identification of anthocyanins from different berries using HPLC-DAD-ESI/MS

| COMPOUND | AGLYCON | SUGAR | Rt (min) | max (nm) | MOL. ION (m/z) | BASE PEAK (m/z) | Highbush Blueberry | Strawberry | Raspberry | Red currant | Blackberry | Black currant |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pelargonidin 3-xylosil-rutinoside | pelargonidin | xylose+rutinose | 13,74 | 501 | 711 | 271,2 | | | X | | | |
| pelargonidin derivative | pelargonidin | | 14,61 | 503 | | 271,2 | | X | | | | |
| pelargonidin derivative | pelargonidin | | 24,21 | 503 | | 271,2 | | X | | | | |
| pelargonidin derivative | pelargonidin | | 25,76 | 503 | | 271,2 | | X | | | | |
| pelargonidin 3-glucoside | pelargonidin | glucose | 12,66 | 501 | 433 | 271,3 | | X | X | | X | |
| pelargonidin 3-malonylglucoside | pelargonidin | glucose | 16,70 | 503 | 519 | 271,2 | | X | | | | |
| pelargonidin 3-rutinoside | pelargonidin | rutinose | 13,99 | 503 | 579 | 271,2 | | X | X | | | X |
| peonidin 3-arabinoside | peonidin | arabinose | 14,45 | not pure | 493 | 331,3 | X | | | | | |
| peonidin 3-galactoside | peonidin | galactose | 12,94 | 517 | 463 | 301,3 | X | | | | | |
| peonidin 3-glucoside | peonidin | glucose | 13,75 | 520 | 463 | 301,3 | X | X | | | | |
| peonidin 3-rutinoside | peonidin | rutinose | 14,55 | 518 | 609 | 301,3 | | | | | | X |
| peonidin 6-acetyl-3-glucoside | peonidin | glucose | 20,00 | 514 | 505 | 301,3 | X | | | | | |
| petunidin 3-arabinoside | petunidin | arabinose | 13,16 | 525 | 449 | 317,3 | X | | | | | |
| petunidin 3-galactoside | petunidin | galactose | 11,55 | 525 | 479 | 317,3 | X | | | | | |
| petunidin 3-glucoside | petunidin | glucose | 12,35 | 526 | 479 | 317,3 | X | | | | | |
| petunidin 3-rutinoside | petunidin | rutinose | 13,30 | 528 | 625 | 317,1 | | | | | | X |
| petunidin 6-acetyl-3-glucoside | petunidin | glucose | 18,49 | not pure | 521 | 317,3 | X | | | | | |

acid, methyl-sanguisorboate, methyl gallate and sanguisorbic acid - were analyzed in 21 raspberry samples (Fig. 2) and 15 blackberry samples (Fig. 2) grown in Trentino (Italy). The concentrations of ellagic acid released during acid hydrolysis ranged from 612 to 1174 mg/kg fresh weight in raspberries and from 576 to 2399 mg/kg fresh weight in blackberries. Values for the second quantitatively most important compound (methyl-sanguisorboate) were between 262 and 487 mg/kg fresh weight for raspberries and between 261 and 1180 mg/kg fresh weight for blackberries. The values for other minor compounds released during hydrolysis (methyl gallate and free sanguisorbic acid), were up to 101 mg/kg in raspberries and up to 182.5 mg/kg fresh weight in blackberries. All four compounds showed extensive variability among cultivars. The average structure of the ellagitannins was highly conserved in the 37 different *Rubus* genotypes, while they differed in the absolute concentration.

The ratio between maximal and minimal ellagitannin concentrations recorded among red raspberry cultivars did not exceed 1.7, indicating relatively limited variability among the cultivars investigated. This variability was much higher in blackberries reaching a ratio of 4.4 between the cultivars ('Adrienne' and 'Apache').

## Conclusions

This finding calls for the necessity to take into account the content of these health-promoting compounds for future breeding programs. An effort in this direction is to be encouraged, considering that the choices of the producers and breeders can, in perspective, affect the health status of the consumers.

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