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Antioxidant capacities and anthocyanin characteristics of the black–red wild berries obtained in Northeast China

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ABSTRACT

Various edible berries widely accessible in nature in Northeast China are poorly exploited. The compositions and contents of anthocyanins in black (*Padus maackii*, *Padus avium*, *Lonicera caerulea*, and *Ribes nigrum*) and red (*Ribes rubrum*, *Sambucus williamsii*, *Rubus idaeus*, and *Ribes procumbens*) wild berries in Northeast China were firstly characterized by HPLC–DAD/ESI-MS^2. Twenty-three anthocyanins were detected and identified. Cyanidin glycosides were dominant in both berries. Six anthocyanins were reported for the first time in *P. avium*, *R. rubrum*, and *Sambucus*. Total anthocyanin content (TAC) ranged from 10 mg/100 g fresh weight (FW) (*R. procumbens*) to 1058 mg/100 g FW (*P. maackii*) among berries. The TACs and antioxidant activities assessed by DPPH and FRAP assays were much higher in black than in red berries. Black–red berries, especially *P. maackii* and *P. avium*, can be used in developing functional foods and in improving breeding programs.

1. Introduction

Berries generally refer to small, brightly colored soft fruits, including strawberries, blueberries, cranberries, and others that are not very important economically (Manganaris, Goulas, Vicente, & Terry, 2014). Berries are highly regarded on account of their attractive colors, unique flavors, delicate textures, and especially their numerous health benefits. Berries have attracted attention worldwide because of their well-known biological activities. Numerous investigations have indicated that consumption of berries can prevent or delay the onset of chronic age-related diseases, e.g., cardiovascular disorder, memory decline, eyesight fading, and cancer, owing to their antioxidant, anti-inflammatory, gastro-protective, and antimicrobial activities (Kong, Chia, Goh, Chia, & Brouillard, 2003; Krikorian et al., 2010; Liu et al., 2011; Zafra-Stone et al., 2007). Recently, increased consumption of berries has been associated with reduced risk of all-cause mortality and mortality related to cancer and stroke (Hjartåker, Knudsen, Tretli, & Weiderpass, 2015). These health-promoting effects of berries are largely attributed to their phenolic compounds; the most abundant of which are the anthocyanins (Zafra-Stone et al., 2007). Nineteen naturally occurring anthocyanidins or aglycones have been detected; six of which are common in higher plants (Iwashina, 2000).

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Berries are the richest sources of natural anthocyanins in many fruits and vegetables. Berries from the genus *Vaccinium* have recently been acclaimed worldwide. *Vaccinium* berries from Italy (Prencipe et al., 2014), Canada (Kalt et al., 2008), Finland (Lätti, Riihinen, & Jaakola, 2011), and America (Grace, Esposito, Dunlap, & Lila, 2014), were systematically investigated. All six common, naturally occurring anthocyanidins, namely, delphinidin, cyanidin, petunidin, peonidin, pelargonidin, and malvidin, were detected in *Vaccinium* berries. The results have also suggested that the wild *Vaccinium* berries apparently possess higher anthocyanin contents and antioxidant activities than their cultivars. Apart from *Vaccinium* berries, other edible berries are also highly valued. For instance, *Rubus idaeus* is a rich source of natural anthocyanins, which is dominated by cyanidin 3-sophoroside (Mazur, Nes, Wold, Remberg, & Aaby, 2014). The production and market of *R. idaeus* have considerably increased in the past two decades. *Lonicera caerulea*, which is a blue-colored berry that looks like blueberry and tastes slightly bitter, is cultivated easily owing to its strong adaptability. Cyanidin 3-glucoside and cyanidin 3-rutinoside are the major anthocyanins in *L. caerulea* berries (Jurikova et al., 2012). *Ribes nigrum*, which is also rich in anthocyanins, is highly tolerant to leaf damage and offers a potential for pesticide-free (organic) farming (Khoo, Clausen, Pedersen, & Larsen, 2012).

These colored berries are exploited and utilized mainly in the United States and among the European countries. By contrast, the wild berry resources in China are diverse and have been rarely reported. Northeast China harbors the largest natural distribution area of wild berries and is the richest in terms of berry species, especially, *Vaccinium uliginosum* of the family Ericaceae. In addition to *V. uliginosum*, *R. idaeus*, *L. caerulea*, and *R. nigrum* as mentioned above, *Padus maackii*, *Padus avium*, *Ribes rubrum*, *Sambucus williamsii*, and *Ribes procumbens* are also abundant in Northeast China. The harvest for *L. caerulea*, *R. nigrum*, and *R. procumbens* in this area reaches 2000, 4000, and 3000 tons per year, respectively (Zhang, Wei, Ma, & Xu, 2006). In addition, all of these berries are medicinal species and have been used as folk herbs, e.g., anti-inflammatory and antidiarrheal tablets or pain killers, by natives. Unfortunately, except for *V. uliginosum*, only a few of the other berries have been harvested and utilized in the past decades. Therefore, the contents and compositions of anthocyanins in these berries remain unknown.

By using HPLC–DAD/ESI-MS^2, the present study systematically characterized the anthocyanins of eight poorly used black–red berries from genera *Padus*, *Lonicera*, *Ribes*, *Sambucus*, and *Rubus*, which are distributed in Northeast China. Furthermore, 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) assay and ferric reducing ability of plasma (FRAP) assay were used to assess the antioxidant activities of the above berries. To the best of our knowledge, this work is the first to report on the anthocyanins of berries from Northeast China, as well as the antioxidant activities of berries. The results of this study will improve the economic utilization of these natural berry resources, as well as accelerate the breeding process. This study will also promote the development of healthy beneficial food products.

2. Materials and methods

2.1. Reagents

Standard cyanidin 3-glucoside was obtained from Extrasynthese (Genay, France). Formic acid, methanol, and acetonitrile used in HPLC–DAD/ESI-MS^2 were of chromatographic grade and were purchased from Alltech Scientific (Beijing, China). Folin–Ciocalteu's phenol reagent, DPPH$^{\cdot}$, and 2,4,6-tripyridyl-*S*-triazine (TPTZ) were purchased from Sigma–Aldrich (St. Louis, USA) and were used to determine antioxidant capacity. Other chemicals, including sodium carbonate, sodium acetate, ferric chloride, methanol, hydrochloric acid, and acetic acid were of analytical grade and were obtained from Beijing Chemical Works (Beijing, China). Double distilled water was produced using a Milli-Q System (Millipore, Billerica, MA, USA).

2.2. Plant materials

Black wild berries (*P. maackii*, *P. avium*, *L. caerulea*, and *R. nigrum*) and red wild berries (*R. rubrum*, *S. williamsii*, *R. idaeus*, and *R. procumbens*) were analyzed (Fig. 1). The berries in their full maturation stage were harvested from the Greater Khingan Mountains, Heilongjiang Province, Northeast China, on a sunny morning of August 2013 (Table 1). The bushes displaying similar morphological characteristics in each location were randomly selected. Approximately 200 g of fresh berries of appropriate size were collected in triplicate for each species. In addition, the distances between the duplicates were more than 100 m. All berry samples were placed in an airtight preserving box containing ice bags and then extracted immediately upon arrival at the laboratory.

2.3. Anthocyanins extraction

Anthocyanins were extracted from the berries according to the method described by Wang et al. (2014) with some modifications. Approximately 2 g of fresh berries were placed in 10 mL centrifuge tubes containing 5 mL 2% formic acid methanol (v/v), and then the mixtures were shaken by a QL-861 vortexer (Kylinbell Lab Instruments, China) for 30 s. The samples were subsequently sonicated in a KQ-500DE ultrasonic cleaner (Ultrasonic instruments, China) at 20 °C for 20 min and then centrifuged using SIGMA 3K30 centrifuge (SIGMA centrifuger, Germany) at 12,000*g* for 10 min. The supernatants were then collected in 50 mL centrifuge tubes. The steps above were repeated until the extracts were colorless. Thereafter, 5 mL extracts were dried using multifunction sample concentrator with nitrogen. The residue was re-dissolved in 1 mL of 2% formic acid methanol (v/v) and then passed through 0.22 μm millipore membrane filters (Shanghai ANPEL, China) prior to the HPLC analyses.

2.4. HPLC–DAD and HPLC–ESI-MS^2 analyses

The conditions for the HPLC–DAD and HPLC–ESI-MS^2 systems were similar to those described in a previous study (Wang et al., 2014). Eluent A was 10% formic acid in double distilled water (v/v), whereas eluent B was 15% methanol in acetonitrile (v/v). The following gradient elution programs were used: for *P. avium* and *P. maackii*, 5% B at 0 min, 12% B at 30 min, 25% B at 50 min, and 5% B at 60 min; for *R. idaeus*, 5% B at 0 min, 12% B at 15 min, 15% B at 20 min, and 5% B at 25 min; for *L. caerulea*, 8% B at 0 min, 25% B at 15 min, and 8% B at 20 min; for *S. williamsii*, *R. rubrum*, *R. nigrum*, and *R. procumbens*, 5% B at 0 min, 8.5% B at 15 min, 10% B at 22 min, 15% B at 35 min, 30% B at 45 min, and 5% B at 50 min. For HPLC–DAD analysis, 10 μL solution of each sample was injected, the flow rate was 0.8 mL min^{-1}, and the column temperature was maintained at 35 °C. Chromatograms were obtained at 525 nm, and photodiode array spectra were recorded at 200–800 nm. For HPLC–ESI-MS^2 analysis, the anthocyanins were analyzed in positive ion (PI) mode, and the MS detection conditions were as follows: capillary voltage, 4.0 kV; nebulization pressure, 241.3 kPa; gas (N_2) temperature, 350 °C; gas flow rate, 8.0 L/min; capillary offset voltage, 77.2 V; capillary exit voltage, 127.3 V; and scan range, 100–1000 (*m*/*z*).