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Comparison of bioactive potential of cranberry fruit and fruit-based products versus leaves

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ABSTRACT

The aim of the study was to compare fruit and fruit-based products (juice and pomace) of large cranberry (*Vaccinium macrocarpon* L.) with leaves as potential sources of health-promoting compounds. The study included leaves and ripe fruit of three cranberry cultivars: 'Stevens', 'Ben Lear', and 'Pilgrim'. The leaves and fruit were analysed for the presence and content of polyphenolic compounds using LC-PDA-MS QTOF and UPLC-PDA-FL methods, and for their antioxidant activity using ABTS, DPPH, and FRAP methods. The fruit, fruit-based products and leaves were found to contain flavonols, flavan-3-ols, phenolic acids, anthocyanins and dihydrochalcone. Regardless of cultivar, the leaves and pomace contained more polyphenolic compounds and exhibited higher antioxidant activity than fruit and juices. Therefore, the pomace and leaves are attractive raw products for the production of foods with high health-promoting value.

1. Introduction

Large cranberry (*Vaccinium macrocarpon* L.) belongs to the family of *Ericaceae*. It is a multiannual, green, low-growing shrub producing red, spherical berries and tiny narrow leaves (Borges, Degeneve, Mullen, & Crozier, 2010; Hołderna-Kędzia, 2006). Cranberry fruits are a rich source of vitamins (A, C, B1, B2, B6, E, lutein and β-carotene), minerals (sodium, potassium, magnesium, iodine, phosphorus, calcium, iron, copper, cobalt, zinc, molybdenum, manganese, aluminum and silver), fibre, sugars (glucose, fructose, sucrose) and organic acids (citric acid, benzoic acid and quinic acid) (Kozłowski, Wielgosz, & Cis, 2007; Rodowski, 2001). The most valuable group of bioactive compounds typical for cranberry fruit are polyphenols exhibiting strong antioxidant properties, including mainly flavonoids such as anthocyanins, flavan-3-ols, flavonols and phenolic acids (Borowska, Mazur, Gadzała-Kopciuch, & Buszewski, 2009; McKay & Blumberg, 2007).

In vitro and animal studies have confirmed very high biological potential of polyphenols typically found in large cranberry (Vattem, Ghaedian, & Shetty, 2005). Cranberry and its products exhibit antifungal, antimicrobial, antipyretic, anticancer, antianemic, and detoxifying properties and are

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extremely rich in vitamins. Cranberry is used in the prevention of rheumatic disorders, tonsilitis, and general weakening, and it is known to improve the functioning of intestines, stomach or pancreas (Kozłowski et al., 2007; Rodowski, 2001). Consumption of cranberry may also reduce the risk of cardiovascular diseases (Vattem et al., 2005).

Due to its characteristic bitter taste, cranberry is usually consumed in a processed form of juices or purees. Fruit pomace that is a leftover of juice production is very valuable and can be dried and used for the production of health promoting or functional foods. In the search for bioactive substances, we decided to investigate cranberry leaves as a possible source of valuable compounds. There are no scientific reports concerning an antioxidant profile of cranberry leaves, even though (Teleszko & Wojdyło, 2015) earlier studies indicate that they are a rich source of polyphenolic compounds. Taking into account a wide range of polyphenol properties, leaves could become a complementary raw product in fruit processing, not only enhancing health-promoting character of food, but also improving and enriching its taste.

The aim of this study was to compare the bioactive compounds of cranberry (*Vaccinium macrocarpon* L.) fruit and fruit-based products such as juice and pomace, with the bioactive substances of leaves of selected cultivars 'Stevens', 'Ben Lear', 'Pilgrim', and to assess these materials as potential sources of health-promoting compounds. Therefore, the raw products (fruits and leaves) and their preparations (juices and pomace) were evaluated (i) for the content and type of phenolic compounds by LC-PDA-QTOF MS and UPLC-PDA-FL, and (ii) for their antioxidant properties (FRAP, ABTS, DPPH).

2. Materials and methods

Plant material consisted of fruit (~4 kg per cultivar) and leaves (~0.5 kg per cultivar) of 'Stevens', 'Ben Lear' and 'Pilgrim' cultivars obtained from a horticultural farm in Wola Mysłowska (51°84′N, 21°93′E), near Warsaw, Poland. Cranberry was grown in sandy loam soil. Nitrogen fertilization in the form of ammonium sulphate (30 kg/ha; $(NH_4)_2SO_4$) was carried out in March and before flowering. In the year preceding fruit harvest, the crop was fertilized with 30 kg P_2O_5/ha as superphosphate, 40 kg K_2O/ha as potassium sulphate, and sulphur 100 kg/ha as VigorS (Siarkopol, Tarnobrzeg, Poland). Average annual temperature until September was 10.4 °C (average temperature for January–March was 1.0 °C, for April–June 12.3 °C, and for July–September 16.6 °C), annual rainfall until September was 570 mm (rainfall amount for January–March was 90 mm, for April–June it was 200 mm, and for July–September it amounted to 170 mm).

The raw material was collected ripe and ready for consumption. The fruits (~1 kg) and leaves were freeze-dried using Alpha 1–4 LSC device (Christ, Osterode, Germany). Then, homogeneous powders were obtained by crushing the dried tissues with a closed laboratory mill to avoid hydration (IKA A.11, Staufen, Germany), and the powder was passed through a strainer (1 mm). The powders were kept in a refrigerator (–80 °C), until 24 h before extract preparation.

2.1. Preparation of cranberry juices on a laboratory scale

Cranberry fruits (3 kg per cultivar) were ground using a Thermomix (Wuppertal, Vorwerk, Germany) laboratory mill for 20 s. After grounding, the mash was pressed under a laboratory hydraulic press (SRSE; Warsaw, Poland), and the juice was heated in Thermomix up to 90 °C for 4 min, hot filled into 0.08 L glass jars, immediately inverted for 10 min to sterilize the lids, and cooled to 20 °C. Two technological replicates of cranberry juice preparation were carried out.

2.2. Qualitative and quantitative of polyphenols

Qualitative (LC/MS QTOF) and quantitative (UPLC-PDA-FL) analysis of polyphenols (anthocyanin, flavan-3-ol, flavonol, and phenolic acid) was performed as described previously by Wojdyło, Nowicka, Laskowski, and Oszmiański (2014). All measurements were repeated three times. The results were expressed as mg per 100 g dry matter (dm) or for juices as mg per 100 ml.

2.3. Analysis of procyanidins by phloroglucinolysis method

An analysis of polymeric procyanidins by phloroglucinol method was performed according to the protocol described previously by Wojdyło et al. (2014). All measurements were repeated three times. The results were expressed as mg per 100 g dm or for juices as mg per 100 ml.

2.4. Determination of antioxidant activity

The solvent from fruits, pomace and leaves for analysis of polyphenols was prepared as described previously by Wojdyło et al. (2014). The ABTS, DPPH and FRAP assay were determined as previously described by Re, Pellegrini, Proteggente, Pannala, and Yang (1999), Yen and Chen (1995) and Benzie and Strain (1996), respectively. All antioxidant activities were expressed as millimoles of Trolox per 100 g dry matter or for juices sample as mmoles of Trolox per 100 ml. Determinations by ABTS and FRAP methods were performed using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan).

2.5. Statistical analysis

Results were presented as mean ± standard deviation of three independent determinations. All statistical analyses were performed with Statistica version 10.0 (StatSoft, Tulsa, USA). One-way analysis of variance (ANOVA) by Duncan's test was used to compare the mean values.

3. Results and discussion

3.1. Identification of phenolic compounds in cranberry fruits and leaves

Identification of polyphenolic compounds in cranberry fruits, juice, pomace and leaves was performed by LC-PDA-ESI-MS/