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Blue honeysuckle (*Lonicera cearulea* L. subs. *edulis*) berry; A rich source of some nutrients and their differences among four different cultivars

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ABSTRACT

Organically grown blue honeysuckle berries have become extremely popular in the last few years, mainly because of their taste and high ascorbic acid content. With the spectrophotometer and aid of high-performance liquid chromatography (HPLC) coupled with mass spectrophotometry (MS) we compare the content of organic and ascorbic acids, sugars, individual phenolics, total phenolics, saponins and tannins, of four blue honeysuckle berry cultivars. On average the cultivar 'Aurora' was the biggest in terms of weight and size, with the highest sugar (2585.45 mg/100 g) and the lowest organic acid (655.46 mg/100 g) contents. Conversely, the cultivar 'Honey Bee' was the smallest and had the highest ascorbic acid (25.77 mg/100 g) and saponin (640.79 mg/100 g), but the lowest sugar (1557.37 mg/100 g) contents. Cultivars 'Borgalis' and 'Tundra' had intermediate weight and size, with high and low contents of other identified compounds.

1. Introduction

The *Lonicera* genus includes approximately 200 species. Most of them grown in the wild but some of them are cultivated. Their trumpet-shaped flowers, with a variety of colours, adorn small gardens and parks as an ornamental bush (Thompson, 2008). Yellow, red or blue berry fruits develop from the flowers. Only the blue honeysuckle species (*Lonicera cearulea* L. subsp. *edulis*), with blue berries, can be eaten (Bors et al., 2012). The edible blue honeysuckle comes from Russia and in the last few years has been considerably planted in some European countries (Slovakia, Poland, Czech Republik, and Slovenia). Its interesting characteristics are high resistance to cold, different soil acidities, pests and various diseases; in short, honeysuckle berries not require special care during cultivation (Hummer et al., 2012; Bors et al., 2012; Thompson, 2008). Their only requirments are pollinators (Hummer et al., 2012) and a sufficient water source (Pokorna-Jurikova and Matuskovic, 2007).

Honeysuckle berries have a more elongated shape than blue-berries. The berries are dark purple with a waxy coting, with elongated elliptic, or cylindrical shape, weighing from 0.3 to 2.0 g, depending on the variety (Hummer et al., 2012; Thompson, 2008). The taste is bitter to sour-sweet, as a mixture of known berry flavours (Hummer et al., 2012). The fruits reach fully maturity early in the season, from mid-May to the beginning of June, what is before strawberries (Bors et al., 2012; Ochmian et al., 2012; Jurikova et al., 2009). The berries are rich in a number of primary and secondary metabolites. They have an extremely high ascorbic acid and phenolic contents (Jurikova et al., 2009; Jurikova et al., 2012), which have nutritional and health promoting properties for humans. Ascorbic acid or vitamin C is one of the most abundant antioxidants in plants (Zheng, 2013). Selected phenolics from honeysuckle berries have antimicrobial, antifungal and anticancer properties (Palikova et al., 2008; Farcasanu et al., 2006; Gruia et al., 2009). Anthoyanins, responsible for antioxidant activity are the biggest contributors to total phenolics in blue honeysuckle berries. Cyanidin-3-glucoside, the main anthocyanin, occurs in dark fruits such as blueberries, chokeberries, black-currants (Wojdyło et al., 2013; Zorenc et al., 2016) and *Mahonia aquifolium* berries (Coklar and Akbulut, 2017). In addition to ascorbic acid and phenolics the *Lonicera* genus also accumulates saponins (Becker et al., 2017) and tannins inside the plant organs. Tannins are responsible for the flavours in various fruits (Wojdyło et al., 2013; Senica et al., 2015) and could be useful food material for treating hypercholesterolemia (Gato et al., 2013). In addition to tannins, iridoids and saponins, compounds commonly found in the *Lonicera* genus have an anti-inflammatory and analgetic effect on human health (Ryu et al., 2010; Yassin et al., 2013; Oszmiański and Kucharska, 2018)

Blue honeysuckle berries are becoming a new functional food and the aim of the study was to compare the weight, size, organic acids, ascorbic acid, sugars, total saponins, total and individual phenolics and total tannins of four blue honeysuckle (*Lonicera cearulea* subs. *edulis*) cultivars. The four cultivars 'Aurora', 'Borealis', 'Honey Bee' and 'Tundra' were collected at the same location to prevent environment and

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pedological factors. The study aimed to discover the most suitable honeysuckle cultivar for farming in Slovenia. Some previous studies have investigated some other honeysuckle cultivars and only the presence of a few plant metabolites. Cultivars from the present study were studied for the first time and for the first time saponin and tannin contents in *Lonicera cearulea* subsp. edulis species were determinated.

2. Materials and methods

2.1. Plant material

Organically farmed honeysuckle berries from four cultivars, 'Aurora', 'Borealis', 'Honey Bee' and 'Tundra', were harvested at the technological ripening stage (1. June 2017) from the Vučetinec location (46°26′22″N; 16°22′15″E; 270 m). Dates were obtained from the Slovenian Environment Agency (ARSO).

2.2. Physical fruit characteristics

For weight determination, fifty berries of each cultivar were randomly selected and weighed on an electrical weighing machine. The size of the berries was measured in terms of length and width with the help use of Vernier callipers. For the determination of dry matter, ten berries in triplicate for each cultivar were dried at 105 °C for 12 h in a drying oven.

2.3. Determination of ascorbic acid

The ascorbic acid content in the samples was determinated according to the method of Mikulic-Petkovsek et al. (2013). Five g of berry paste was extracted with 10 ml of 2% *meta*-phosphoric acid. Each sample was left at room temperature for 1 h on a shaker (Grant-Bio POS-300, Grant Instruments, Shepreth, England). The samples were then centrifuged (Eppendorf 5810 R Centrifuge, Hamburg, Germany) at 4 °C at 10 000 rpm for 7 min and filtered through a Cromatofil A-20/25 mixed ester filter (Macherey-Nagel, Düren, Germany) into vials until further analysis. Determination of ascorbic acid was carried out with the Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA). Conditions were the same as for sugars and organic acids, which were previously described by Mikulic-Petkovsek et al. (2012). The contents were expressed in mg of ascorbic acid per 100 g fresh weight.

2.4. Determination of sugars and organic acids

Sugar and organic acids in the different honeysuckle cultivars were estimated according to the method of Mikulic-Petkovsek et al. (2012). 5 g of fresh honeysuckle berries were blended with an Ultra-Turrax T-25 macerator (Ika-Labortechnik, Stauden, Germany) and extracted with 25 ml double-distilled water. Each sample was left at room temperature on a shaker for 30 min. The samples were then centrifuged at 4 °C at 10 000 rpm for 7 min and filtered through Cromatofil A-20/25 cellulose mixed ester filters into vials and stored for further analysis. Determination of individual sugars and organic acids was carried out with the Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) according to the method reported by Mikulic-Petkovsek et al. (2012).

2.5. Determination of individual phenolics

Extraction of phenolic compounds from the four different honeysuckle cultivars was carried out as described by Senica et al., (2015) with some modifications. Berries were homogenized with an Ultra-Turrax T-25 and 5 g of fruit paste was extracted in 30 ml-centrifuge tubes with 15 ml methanol containing 3% formic acid, and placed in into a cool ultrasonic bath for 1 h. The extracts were subsequently centrifuged for 10 min at 12-000 rpm. Each supernatant was filtered through a Chromafil AO-20/25 polyamide filter (Macherey-Nagel, Düren, Germany) and transferred into vials for further HPLC and MS analysis. Separation of phenolic compounds was performed on a mass spectrometer (LCQ Deca XP MAX, Thermo Scientific) with electrospray ionization (ESI) operated in negative ion mode. The ESI parameters were as described by Senica et al., (2015). Analyses were carried out using the Accela HPLC system (Thermo Scientific, San Jose, CA), equipped with a diode array detector (DAD), controlled by CromQuest 4.0 chromatography workstation software, with technical characteristics as described by Senica et al., (2015), with the mobile phase gradient according to Wang et al. (2002).

2.6. Determination of total phenolic content and total tannins

The extraction of berry samples for the determination of total phenolics was done in the same way as for individual phenolics. Total phenolic content was determinated using the Folin-Ciocalteu phenol reagent method (Wang et al., 2002). Into 100 µl of sample extracts (diluted 1:2 with MeOH) were added 500 µl Folin-Ciocalteu reagent (Singleton et al., 1999), 1.5 ml 20% sodium carbonate and 7.9 ml of double-distilled water. Extracts were vortexed and stood in an ultrasonic bath at 40 °C for 30 min. Total phenolic content was assessed with a spectrophotometer (PerkinElmer, UV–vis Lambda Bio 20) at 765 nm. A mixture of water and other reagents was used as blank. Total phenolic content was expressed as gallic acid equivalents (GAE) in mg/100 g fresh weight of honeysuckle fruit.

The tannin content in each sample was determined using insoluble polyvinylpolpyrrolidine (PVPP), estimated by the method given by Makkar et al., (1993). To bind the tannins, 0.1 g was mixed with 400 µl of diluted sample (1:2 with methanol, v/v). Samples were centrifuged at 10-000 rpm for 5 min. Non-tannin phenolics were measured with a spectrophotometer in the same way as total phenolics content. Tannin content was calculated as the difference between total phenolics content and non-tannin phenolic content in the extract. The results are expressed as mg of gallic acid equivalents (GAE) in 100 g fresh weight.

2.7. Determination of total saponin contents

For quantification of total saponins the honeysuckle berries were first mixed with Ultra-Turrax T-25 and 1 g of fruit paste was extracted with 8 ml of 96% ethanol. The mixture was heated in a drying oven at 60° for 2 h and centrifuged at 4 °C at 10,000 rpm for 20 min. Further analysis was then performed according to the method of Hiai et al. (1976). To 100 µl of sample (diluted 1:4, v/v) was added 0.2 ml vanillin, 0.25 ml ethanol and 2.5 ml 72% H_2SO_4. Samples were placed in an ultrasonic bath and heated at 60 °C for 10 min. After cooling, absorbance the mixture read with a spectrophotometer (PerkinElmer, UV–vis Lambda Bio 20) at 544 nm. Total saponin content was expressed as diosgenin equivalents (DSE) in mg/100 g fresh weight of honeysuckle berries.

2.8. Reagents and standards

From Sigma–Aldrich Chemie (Steinheim, Germany) we purchased following standards: quiniq acid, shikimic acid, chlorogenic acid (5-caffeoylquinic acid), neochlorogenic acid (3-caffeoylqinic acid), cyanidin-3-*O*-glucoside, ellagic acid, and luteolin-3-*O*-rutinoside, diosgenin and ascorbic acid. Furthermore we also purchased from Sigma Aldricht Chemie chemicals such as polyvinylpolpyrrolidine to bind tannins, Folin-Ciocalteu reagent, ethanol for saponin and metaphosphoric acid for vitamin C extraction and methanol for phenolics extraction and additionaly for mobile phases in mass spectrophotometer and high-performance liquid chromatography. From Fluka Chemie (Buchs, Switzerland) we obtained fructose, glucose, sucrose, citric, malic, fumaric and tartatic acid from sugars and organic acids; epicatechin,

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