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Biochemistry

Frost decreases content of sugars, ascorbic acid and some quercetin glycosides but stimulates selected carotenes in *Rosa canina* hips

Vlasta Cunja*, Maja Mikulic-Petkovsek, Anka Zupan, Franci Stampar, Valentina Schmitzer

Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, Ljubljana, SI-1000, Slovenia

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SUMMARY

Primary and secondary metabolites of *Rosa canina* hips were determined by HPLC/MS during ripening and after frost damage. Rose hips were harvested six times from the beginning of September until the beginning of December. Color parameters a^*, b^* and L^* decreased during maturation. Glucose and fructose were the predominant sugars representing up to 92% total sugars, and citric acid was the major organic acid detected in rose hips (constituting up to 58% total organic acids). Total sugar and ascorbic acid content significantly decreased after frost damage; from 42.2 to 25.9 g 100 g^{-1} DW for sugars and from 716.8 to 176.0 mg 100 g^{-1} DW for ascorbic acid. Conversely, β-carotene and lycopene levels increased in frostbitten rose hips to 22.1 and 113.2 mg 100 g^{-1} DW, respectively. In addition to cyanidin-3-glucoside (highest level in hips was 125.7 μg 100 g^{-1} DW), 45 different phenolic compounds have been identified. The most abundant were proanthocyanidins (their levels amounted up to 90% of total flavanol content) and their content showed no significant differences during maturation. The levels of catechin, phloridzin, flavanones and several quercetin glycosides were highest on the first three sampling dates and decreased after frost. Antioxidant capacity similarly decreased in frostbitten rose hips. Total phenolic content increased until the third sampling and decreased on later samplings.

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Introduction

Rosa canina L. is a widespread shrub native to Europe and western Asia. It is a common species of dog roses (*Rosa* sect. *Caninae*), a section of *Rosa* most widespread in Central Europe (Wissemann et al., 2006). For centuries rose hips (pseudofruits) of *R. canina* have been used for nutrition, medicinal purposes and their ornamental value. Hips are rarely eaten fresh; usually they are dried and/or processed and consumed in form of tea, nectar, wine or marmalade (Uggla et al., 2005; Yildiz and Alpaslan, 2012).

Rose hips contain significant amounts of biologically active compounds and are considered a particularly rich source of ascorbic acid (Hvattum, 2002; Roman et al., 2013). Vitamin C functions in activation of enzymes, oxidative stress reduction and demonstrates an important immune function. Vitamin C supplementation is known to have a protective effect against several disease conditions, most notably the common cold, cardiovascular disease and some cancers (Schlueter and Johnston, 2011). The antioxidative effects of *R. canina* have not only been ascribed to vitamin C, but also to polyphenolics (Daels-Rakotoarison et al., 2002; Tumbas et al., 2012). The results of several studies indicate that rose hips possess anti-inflammatory properties and might as such be used as a replacement or supplement for conventional drug therapies in some inflammatory diseases such as arthritis (Daels-Rakotoarison et al., 2002; Rein et al., 2004; Winther et al., 2005). Ninomiya et al. (2007) have also observed that aqueous acetone extracts from spurious fruit and seeds of *R. canina* substantially inhibit the gain of body weight and/or weight of visceral fat in mice. Zocca et al. (2011) recommended a potential use of dog rose hip extract in food industry as an anti-browning agent, since it effectively inhibits polyphenol oxidase and tyrosinase activity.

The distinct orange to red color of rose hips is formed as a result of various carotenoids. The most abundant are β-carotene and lycopene, followed by β-cryptoxanthin, rubixanthin, zeaxanthin and lutein (Hodisan et al., 1997; Hornero-Méndez and Mínguez-Mosquera, 2000; Andersson et al., 2011). Carotenoids are an important part of a healthy human diet, as they function as provitamin A and are supposed to prevent certain chronic diseases and even cancer (Mayne, 1996).

Different species of *Rosa* vary in date and duration of hip maturation. In contrast to some other species of small fruit (e.g.

* Corresponding author. Tel.: +386 1320 3143; fax: +386 14231088.
E-mail address: vlasta.cunja@bf.uni-lj.si (V. Cunja).

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blackberries, raspberries), rose hips do not abscise from branches when ripe. In order to assure an optimal composition of fruits it is important to know how the content of bioactive compounds changes during fruit maturation. An old Slovenian tradition claims that one is supposed to harvest rose hips after they have been subjected to frost. But do cold temperatures really positively affect the content of bioactive compounds in rose hips? The aim of the present study is to, for the first time, identify and quantify different primary and secondary metabolites in *R. canina* hips during ripening and analyze the impact of frost damage on rose fruit composition. This will provide better information to determine the right harvesting period to optimize the content of bioactive compounds and primary metabolites in *R. canina* hips.

Materials and methods

Plant material

Rosa canina rose hips were harvested from bushes growing in nature in the vicinity of Biotechnical Faculty (central Slovenia, lat. 46,18°N, long. 14,61°E, altitude 250 m) every two weeks, from the beginning of September until the beginning of November and once again in early December when they were subjected to frost. All together there were six sampling dates. For each harvest period, a mixed sample of approximately 75 hips was collected. Plants were identified by morphological key characteristics described in the Mala flora Slovenije (Martinčič et al., 2007). Voucher specimens are deposited in the herbarium of the Chair for Fruit, Wine and Vegetable Growing, Department of Agronomy, Biotechnical Faculty, University of Ljubljana. The material was transported to the laboratory facility and seeds (achenes) and calyces were removed prior to the analysis. The analysis for vitamin C and carotenoid content was performed immediately on fresh pericarp. At this point samples for dry weight analysis were taken. The rest of the sample was frozen in liquid nitrogen and stored at −20 °C until further analysis of phenolic compounds, organic acids and sugars.

Rose hip color measurements

Rose hip color was measured by a portable colorimeter (CR-10 Chroma; Minolta, Osaka, Japan) with C illuminant. The colorimeter was calibrated with a white standard calibration plate before use. In CIE L^* a^* b^* system of color representation, the L^* value corresponds to a dark-bright scale and represents the relative lightness with a range from 0 to 100 (0 = black, 100 = white). Color parameters a^* and b^* extend from −60 to 60; a^* negative is for green and a^* positive is for red and b^* negative is for blue and positive for yellow. The hue angle (h°) is expressed in degrees from 0 to 360, where 0° = red, 90° = yellow, 180° = green and 270° = blue. 30 rose hips were measured per sampling date.

Dry matter content determination

Fresh weight (FW) was recorded immediately after harvest after seed removal. Dry weight (DW) was recorded after drying at 105 °C for 48 h in an electrical oven until constant weight was achieved. Five replicates per sampling date were taken, each included approximately five hips. Dry matter (DM) content was determined as the percentage of total pericarp weight (DW × 100/FW) by weighing samples before and after drying.

Determination of sugars, organic acids and vitamin C using high-performance liquid chromatography (HPLC)

Extraction of sugars and organic acids was carried out as reported by Mikulic-Petkovsek et al. (2012) with some modifications. A mixed sample of rosehip pericarp (five replicates per sampling date) was ground to a paste in a mortar and 2.5 g were extracted with 12.5 mL of double distilled water. Extraction of vitamin C was carried out using the same procedure except that 1 g of the paste was extracted with 5 mL of 2% metaphosphoric acid. Samples were left at room temperature for 30 min on an orbital shaking platform (Grant-Bio POS-300, Grant Instruments, Shepreth, England), centrifuged (Eppendorf 5810 R Centrifuge, Hamburg, Germany) at 12,000g_n for 5 min at 4 °C and filtered through a Cromafil A-20/25 cellulose mixed ester filter (Macherey-Nagel, Düren, Germany) into vials. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA). Chromatographic conditions used for determination of sugars and organic acids were the same as described by Mikulic-Petkovsek et al. (2012). The chromatographic conditions for ascorbic acid determination were the same as for the organic acids, only the column temperature was set at 20 °C, and the UV detector at 245 nm. Quantification was assessed from peak areas and calculated by the use of a calibration curve of corresponding standards. Concentrations were expressed on a dry weight basis in g or mg per 100 g of pericarp. From the data of individual sugars and individual organic acids, the sums of sugars (total sugars) and organic acids (total acids) were calculated.

Extraction and determination of phenolic compounds using high-performance liquid chromatography coupled with mass spectrometry (HPLC/MS)

The extraction of phenolic compounds from rose hips was carried out as described by Veberic et al. (2014) with some modifications. Rose hips were ground in a mortar (combined samples of five rosehips in five repetitions per sampling date) and 2.5 g of paste was extracted with 4 mL of methanol containing 3% (v/v) formic acid in an ultrasonic bath for 1 h. Samples were centrifuged for 7 min at 12,000g_n. The supernatant was filtered through a Chromafil AO-20/25 polyamide filter (Macherey-Nagel, Düren, Germany) and transferred to a vial prior to injection into the HPLC system. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 280 nm (phenolic acids and their derivatives, flavanols), 350 nm (flavones, flavonols, flavanones, phloridzin and ellagic acid pentosides) and 530 nm (anthocyanins). The equipment and chromatographic conditions used for HPLC analysis were the same as described by Veberic et al. (2014). Phenolics were further identified using a mass spectrometer (LCQ Deca XP MAX; Thermo Scientific) with an electrospray ionization interface (ESI). For detailed mass spectrometry conditions, see Veberic et al. (2014). The identification of compounds was confirmed by comparing retention times and their spectra as well as by adding the standard solution to the sample and by fragmentation. The content of phenolic compounds was assessed from peak areas and quantified with the use of corresponding external standards.

For compounds lacking standards, quantification was carried out using similar compounds as standards. Thus methylgallate hexoside was quantified with the calibration curve of gallic acid, proanthocyanidins and their glycosides by procyanidin B2, catechin hexoside by catechin, coumaroylquinic acids and *p*-coumaric acid hexoside by *p*-coumaric acid, sinapic acid hexoside by sinapic acid. All apigenin derivatives were quantified in equivalent of apigenin-7-glucoside, naringenin and eriodyctiol hexosides by naringenin, ellagic acid pentosides, methyl gallate acetyl dihexoside, methyl gallate rutinoside and methyl ellagic acid pentoside by ellagic acid, isorhamnetin hexosides by isorhamnetin-3-glucoside and quercetin-3-galactoside by calibration curve of quercetin-3-galactoside. Total flavonols, flavanons, proanthocyanidin (PA) dimer glycosides, PA aglycons, PAs, flavanols and total

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