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Autochthonous Rose Hybrid *Rosa pendulina* × *spinosissima* Overshines Main Genotype *Rosa pendulina* in the Biochemical Characteristics of Their Hips

Nina Kunc [1], Maja Mikulič-Petkovšek [1], Metka Hudina [1], Jože Bavcon [2], Branko Vreš [3], Gregor Osterc [1,*] and Blanka Ravnjak [2]

[1] Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia; ninakunc123@gmail.com (N.K.); maja.mikulic-petkovsek@bf.uni-lj.si (M.M.-P.); metka.hudina@bf.uni-lj.si (M.H.)
[2] University Botanical Garden, Biotechnical Faculty, University of Ljubljana, Ižanska cesta 15, 1000 Ljubljana, Slovenia; joze.bavcon@guest.arnes.si (J.B.); blanka.ravnjak@bf.uni-lj.si (B.R.)
[3] Jovan Hadži Institute of Biology SRC SASA, Zagorica 20, 1292 Ig, Slovenia; brane.vres@zrc-sazu.si
* Correspondence: gregor.osterc@bf.uni-lj.si

Abstract: The medicinal value of rose hips largely depends on the contents of vitamin C and flavonoids. Rose hips contain more vitamin C than most fruits and vegetables. We were particularly interested in how the bioactive substances of rose hips are inherited from main rose species. The aim of the study was to compare the contents of ascorbic acid, organic acids, and phenolics in the fruits, rose hips, of *Rosa pendulina* L. and the hybrid *Rosa pendulina* L. × *spinosissima* L. The contents of ascorbic acid in the studied hips were higher in *R. pendulina* × *spinosissima* than in *R. pendulina*. The contents of total organic acids were higher in the hips of *R. pendulina* × *spinosissima* than in the hips of *R. pendulina*. The contents of phenolics in the hybrid were significantly higher in flesh with skin and in seeds. The contents of cyanidin-3-glucoside were higher in *R. pendulina* × *spinosissima*. We can conclude that the contents of bioactive substances in our samples is rich and could potentially be used for human consumption. The results will help to increase transparency about which rose species provide rose hips that are the most suitable for nutritional purposes.

Keywords: *Rosa pendulina*; *Rosa pendulina* × *spinosissima*; biochemical compounds; rose hips

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1. Introduction

The genus *Rosa* has been known since ancient times. It numbers more than 200 species of roses, which are very widespread throughout the world. Most of them are in Central Asia and Europe. Roses are very diverse in species. They are in all geographical regions of Slovenia, which further affects their diversity. In Slovenia, where the diversity of species of different genera is widespread, only 24 species of wild rose grow, the most important being *R. canina* L., *R. gallica* L., *R. pendulina* L., *R. glauca* L., *R. rubiginosa* L., *R. sempervirens* L., *R. arvensis* Huds., and *R. spinosissima* L. They grow in very extreme habitats, which allows them to adapt well to pioneering conditions [1–3].

Rose hips are rich in polyphenolics (flavonoids, proanthocyanidins, and catechins), triterpenic acids, essential fatty acids, galactolipids, folates, vitamins A, C, and E, minerals (Ca, Mg, K, S, Si, Se, Mn, and Fe), and other bioactive compounds. The extracts have antioxidant, anti-inflammatory, immunomodulatory, anti-cancer, cardioprotective, antidiabetic, neuroprotective, and antimicrobial properties. They have been confirmed to be beneficial against liver disease, osteoarthritis, rheumatoid arthritis, obesity, cancer, kidney stones, depression, and skin problems, among other pathologies. In addition, they also contain large amounts of carotenoids [4]. In general, the large variability in the contents of all biochemical substances is due to genetic, physiological, and environmental influences [5].

Various studies have dealt with the ascorbic acid content in hips of *R. spinosissima*. The importance and large amounts of vitamin C have already been reported by Darlington [6], Uggla [7], and Babis and Kucharska [8]. Oprica et al. [9] hypothesized that the ascorbic acid contents are highly variable. The finding was confirmed by an analysis of roses (*R. spinosissima* and *R. pedndulina*) growing at different altitudes. The influence of altitude was also studied by Roman et al. [3]. It has been reported that changes in ascorbic acid content can be influenced by different altitudes, ecological factors, species, varieties, and harvest times. Turkben et al. [10] also reported that a decreased vitamin C content was due to the amount of oxygen in the environment. The reduction in vitamin C content also depends on the drying technique, temperature, and humidity. Demir et al. [11] reported that, in addition to ascorbic acid, citric acid and malic acid are characteristic organic acids of rose fruits. The same findings were made by Murathan et al. [12]. Demir et al. [11] reported that the contents of phenolic compounds were significantly influenced by the species (*R. canina* L., *R. dumalis* Bechst., *R. gallica*, *R. dumalis* subsp. *boissieri,* and *R. hirtissima* Lonacz). Adamczak et al. [13] compared the flavonoid contents of 11 *Rosa* species (*R. agrestis* Savi., *R. canina*, *R. dumalis*, *R. glauca*, *R. inodora* Fr., *R. jundzillii* Besser, *R. rubiginosa*, *R. sherardii* Davies., *R. tomentosa* Sm., *R. villosa* L., and *R. zalana* Wiesb.) and found that their contents were similar in all examined species. They reported a low level of flavonoids in *R. canina* and the highest content in *R. rubiginosa* hips.

Hybridization is very common in the genus *Rosa*. *R. spinosissima* is a very variable species. There are more than 500 cultivars derived from *R. spinosissima*, more than 200 from *R. galica*, 150 from *R. centifolia* L., 100 from *R. damascena* Mill., and 50 from *R. alba* L. It also forms hybrids with other wild *Rosa* species, and such hybrids may occur in wild communities of *R. spinosissima*. All variants and other cultivars of *R. spinosissima* became known in Britain as Scots or Scotch Roses. Some Scots Roses that were raised in gardens were probably hybrids with non-native species that flowered at the same time of year [14].

Some research has been conducted on the contents of bioactive substances in rose hips and on hybridization in general, but very little is known about the role of hybridization in the contents of bioactive substances in rose hips. The purpose of this work was to analyze, in detail, two genotypes of Rosa and to study the influence of hybridization on the contents of some bioactive compounds in rose hips, separately for pulp and seeds. We also tried to determine the possible influence of crosses on the contents of bioactive substances and whether the hybrids are a sufficient source of these compounds for potential use in human nutrition. We focused on detailed analyses of the contents of ascorbic acid, organic acids, and individual polyphenols in *R. pendulina* and the hybrid *R. pendulina* × *spinosissima*. We assume that in spontaneous hybrids in nature a heterosis effect regarding the contents of various biochemical compounds is probably present, which results in higher contents of various substances in the fruits of these hybrids. Therefore, we assume that the contents of various bioactive substances in the hips of the naturally growing hybrid will be higher compared to the contents of these substances in the hips of the original rose species. In addition, we also assume that the contents of phenolic compounds in the flesh with skin of all analyzed rose hips are higher compared to the contents in the seeds.

2. Materials and Methods

2.1. Plant Material

Rose hips from *R. pendulina* and its hybrid with *R. spinosissima* were collected in 2020 from rose bushes growing in Čaven above the Vipava Valley (Western Slovenia). All plants were grown under the same climatic conditions approximately 15 m apart. All plants included in the experiment were grown in an area of approximately 150 m^2. *R pendulina* grew in the shady edge of the forest, *R. spinosissima* grew over a stone wall, where it was exposed to sun and wind, and the hybrids were in the middle between them, among rocks on the edge of the forest, which were half exposed to the sun during the day. The exact identification of the species was carried out previously with a precise morphological analysis according to the keys for determining the species of *Rosa* L. In

addition to flowers, hips and two-year-old twigs with spines were also used to determine the species. The identification procedure focused on the size of the bush itself, the leaf surface (leathery, shiny, surface color, size, hairiness, and glands), sepals (simple, divided, and shape after flowering), fruits (size, color, shape, and the reverse of the stalks), flower necks (hairiness and neck color), flowers (color), pedicel (length and glands), spines (size, frequency, single/clustered, and shape), and twigs (color and hairiness) (Bavcon et al. [1]). *R. pendulina* hips were collected from a single bush, while the hips of the hybrid plant were collected from three bushes in September 2020. We also tried to collect hips from *R. spinosissima,* but unfortunately, bushes of this species were without hips in autumn 2020. The sampling of the material took place by randomly picking hips from each bush and storing them in plastic bags on which we clearly marked the locations of the fruits. These plastic bags were stored in a cooler bag during transport to the laboratory. In the laboratory, we immediately stored them in the freezer at −20 °C, where the hips waited until the analyses of the phenolic compounds. We did not store the parts of the fruits used for the ascorbic acid and organic acid analyses in the freezer because the analyses were performed immediately on fresh hips. The material was stored at −20 °C until further analysis.

2.2. Extraction and Analysis of Ascorbic Acid

The ascorbic acid extraction was carried out on the rose hips (flesh with the skin and without seeds), as previously described by Mikulic-Petkovsek et al. [15]. First, 15 mL of 3% meta-phosphoric acid was added to 0.5 g of material. The samples were left at room temperature for 30 min on an orbital shaker platform (Unimax 1010, Heidolph Instruments, Schwabach, Germany), centrifuged (Eppendorf 5810 R Centrifuge, Hamburg, Germany) at 10,000× *g* for 5 min at 4 °C, and filtered through a Cromafil A-20/25 cellulose mixed ester filter (Macherey-Nagel, Düren, Germany) into vials. The vials with extracts were stored at −20 °C until further analysis. The samples were analyzed using the HPLC system (Vanquish UHPLC, Thermo Fisher Scientific) and a UV detector set at 245 nm. The chromatographic conditions for ascorbic acid determination were the same as reported by Mikulic-Petkovsek et al. [15]. For the separation of ascorbic acid, we used a Rezex ROA column (Phenomenex) operated at 20 °C with 4 mM sulfuric acid for the mobile phase. The concentrations of vitamin C in fresh weight (FW) were expressed as mg of ascorbic acid/100 g of fresh hips (without seeds). Information related to the standards we used is shown in Table A1 in Appendix A.

2.3. Extraction and Analysis of Organic Acids

First, we separated the seeds from the flesh with skin and discarded them. Sample extraction for organic acid quantification was carried out by the protocol reported by Mikulic-Petkovsek et al. [15]. The flesh with skin was finely chopped. Then, 1 g of material was weighed into centrifuge tubes, and 3 mL of bidistilled water was added. The tubes were placed on a shaker (Unimax 1010, Heidolph Instruments, Germany) for half an hour. The extract was then centrifuged (Eppendorf centrifuge 5810 R) at 10,000× *g* for 7 min at 4 °C. The supernatant was filtered through a syringe filter (Chromafil Xtra MV-20/25, Macherey Nagel, Düren, Germany) into a vial, labeled, and stored at −20 °C. The extraction was performed in triplicate. For organic acid separation, we used a UV detector set at 210 nm, a Rezex ROA column (Phenomenex, USA) heated to 65 °C, and the mobile phase was 4 mM sulfuric acid with a flow rate of 0.6 mL/min. Information related to the standards we used is shown in Table A1.

2.4. Extraction and Analysis of Phenolic Compounds

The extraction of rose hips was performed for flesh with skin and seeds separately using the extraction method reported by Mikulic-Petkovsek et al. [16]. The analyses were performed in triplicate. Each sample was ground with liquid nitrogen in a mortar, a measured weight of the sample was placed in a centrifuge tube, and the extraction solution was added (3% formic acid in methanol with bidistilled water). The ratio of weighed

sample and extraction solution was 1:5. The masses of samples ranged from 0.2 g to 1 g, depending on the amount of collected material, and the volume of the extraction solution was between 1 and 5 mL. Information related to the standards used is shown in Table A1.

Extraction was then carried out in a cooled ultrasonic bath (Iskra PIO, SONIS 4 GT) on ice for 1 h, after which the extract was centrifuged (Eppendorf centrifuge 5810 R) at 10,000× *g* for 7 min at 4 °C. The supernatants were filtered through a 0.20 mm polyamide/nylon filter (Macherey–Nagel, Düren, Germany). Vials with extracts were stored at −20 °C until further analysis of phenolic compounds.

The analysis of phenolic components was performed on the Thermo Scientific Dionex HPLC system with a diode array detector (Thermo Scientific, San Jose, CA, USA) connected to Chromeleon workstation software. The chromatographic method for phenolic analysis was previously described by Mikulic-Petkovsek et al. [16]. The detector was set to three wavelengths: 280 nm, 350 nm, and 530 nm. The mobile phases were A: 3% acetonitrile/0.1% formic acid/96.9% bidistilled water and B: 3% water/0.1% formic acid/96.9% acetonitrile. The gradient elution of both mobile phases was described in Mikulic-Petkovsek et al. [17], and their flow was 0.6 mL/min. The column used was a Gemini C18 (150 × 4.6 mm 3 µm; Phenomenex, Torrance, CA, USA) heated to 25 °C.

Phenolic compounds were identified using a mass spectrometer (LTQ XL Linear Ion Trap Mass Spectrometer, Thermo Fisher Scientific, Waltham, MA, USA) with electrospray ionization (ESI) operating in positive (anthocyanins) or negative (all the other phenolics) ionization modes. All mass spectrometer conditions were set as reported by Mikulic-Petkovsek et al. [17]. Spectral data were elaborated using Excalibur software (Thermo Scientific). The identification of compounds was confirmed by comparing their retention times and spectra, by adding the standard solution to the sample, and by fragmentation and comparison with the literature data.

The contents of phenolic compounds were calculated from the peak areas of the samples and the corresponding standards. The contents were expressed as mg/kg fresh weight (FW) [16].

2.5. Statistical Analysis

The results were analyzed with the R commander statistical program using a one-way analysis of variance (ANOVA) and with Microsoft Excel 2016. The Duncan test was used to compare treatments when the ANOVA showed significant differences among values. The results are given as mean values with standard deviations (SD). If the *p*-values were lower than 0.05, then the differences among genotypes were statistically significant.

3. Results

3.1. Asorbic Acid

The content of ascorbic acid in *R. pendulina* × *spinosissima* hips was 1045.0 mg/100 g FW, which is almost two times more than in *R. penduline*, with 530.0 g/100 g FW (Figure 1).

3.2. Organic Acids

The organic acids determined in rose hips were citric, malic, quinic, shikimic, and fumaric acid. The highest level of total organic acids was found in the hybrid *R. pendulina* × *spinosissima*, 62.83 g/kg FW, while *R. pendulina* had only 29.15 g/kg FW. The high content of organic acids in *R. pendulina* × *spinosissima* is due to the significantly higher contents of quinic (48.8 g/kg FW) and citric acid (13.6 g/kg FW). Malic acid was not present in *R. pendulina* × *spinosissima*. Shikimic and fumaric acid were present in the hips of both genotypes in the lowest amounts (Table 1).

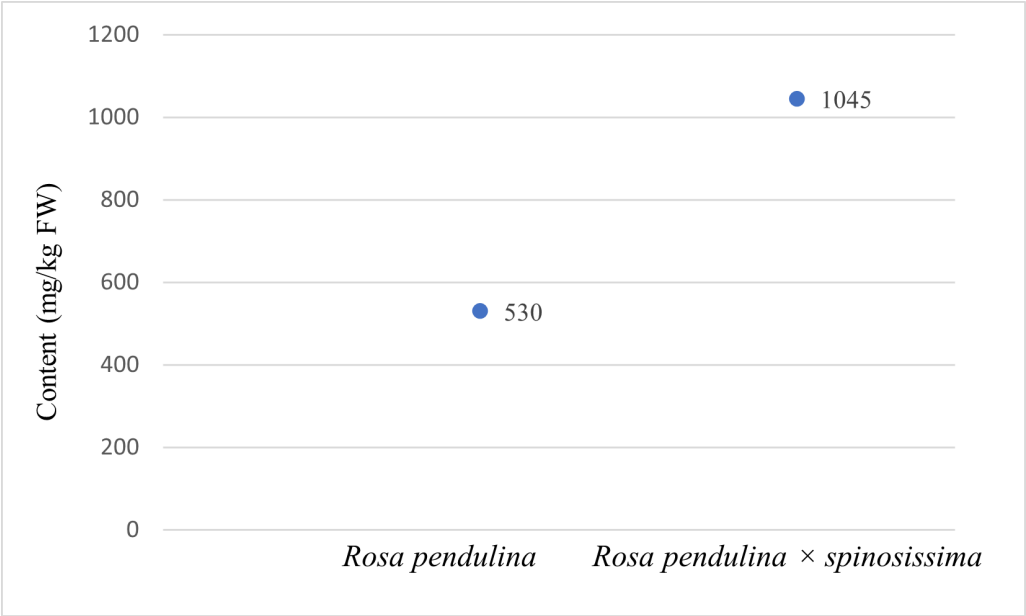


Figure 1. Comparison of the average contents (mg/100 g FW) of ascorbic acid in hips of *R. pendulina* and the hybrid genotype *R. pendulina* × *spinosissima* in the region of Western Slovenia in 2020. Mean values with corresponding standard deviations are presented. Different letters attribute a statistical difference between the average values of ascorbic acid contents in the hips of *R. pendulina* and *R. pendulina* × *spinosissima*.

Table 1. The average contents ± standard deviations (mg/kg FW) of organic acids in hips of *R. pendulina* and the hybrid genotype *R. pendulina* × *spinosissima* in the region of Western Slovenia in 2020. Different letters signify a statistical difference between the average values of organic acid contents in the hips of *R. pendulina* and those of *R. pendulina* × *spinosissima*.

Compound	*Rosa pendulina*	*Rosa pendulina* × *spinosissima*
citric acid	1278.7 ± 316.8 b	13,611.4 ± 3829.5 a
malic acid	1329.2 ± 541.7	-
quinic acid	296.6 ± 93.5 b	48,769.3 ± 28,824.0 a
shikimic acid	8.1 ± 0.6 b	85.1 ± 4.6 a
fumaric acid	2.6 ± 0.5 b	365.9 ± 1.8 a
TOTAL	2915.2 ± 953.1 b	62,831.7 ± 32,659.9 a

3.3. Phenolic Compounds

In the analyzed *Rosa* genotypes, 61 different phenolic compounds were identified. The content of the total analyzed phenolic compounds was two times higher in *R. pendulina* × *spinosissima* hips (flesh with skin) than in *R. pendulina* hips. The phenolic contents in the seeds were quite similar in both genotypes but slightly higher in the hybrid (Figure 2).

The total hydroxybenzoic acid (HBA) derivative content was significantly higher in *R. pendulina* (flesh with skin 1151.0 mg/kg FW and seed 980.7 mg/kg FW) than in *R. pendulina* × *spinosissima* (flesh with skin 2336.5 mg/kg FW and seed 189.7 mg/kg FW). This was true for both flesh and seeds. From the group of hydroxycinnamic acids, five p-coumaric acid derivatives, two synapic acid derivatives, and two caffeic acid derivatives were found. The hybrid *R. pendulina* × *spinosissima* was characterized by an extremely high content of total hydroxycinnamic acids. This was true for both the pulp (990.6 mg/kg FW) and the seeds (222.5 mg/kg FW).

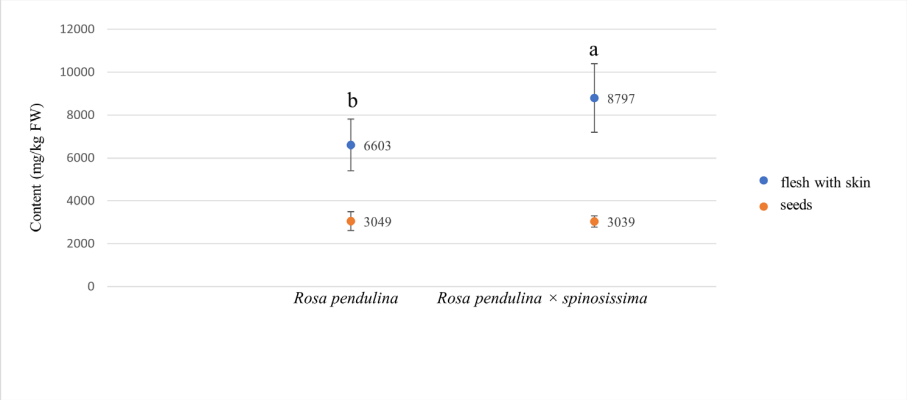


Figure 2. Comparison of total analyzed phenolic contents (mg/kg FW) in flesh with skin and seeds of *R. pendulina* hips and the hybrid genotype *R. pendulina* × *spinosissima* in the region of Western Slovenia in 2020. Mean values with corresponding standard deviations are presented. Different letters signify statistical differences between the two genotypes. The comparison between genotypes was made separately for seeds and for flesh with skin.

The contents of gallotannins and ellagitannins were also higher in the *R. pendulina* × *spinosissima* hybrid (Table 2). In the fruit flesh with skin, the content of gallotannins in *R. pendulina* × *spinosissima* was 387.1 mg/kg FW and 121.5 mg/kg FW in the seeds of the hybrid. The content of gallotannins in *R. pendulina* was 169.1 mg/kg FW in flesh with skin and 41.6 mg/kg FW in seeds. The same picture was found for ellagitannins, where the flesh of the hybrid had a more than two times higher content and the seeds had an almost two times higher content of ellagitannins than *R. pendulina* (Table 2).

Table 2. The contents ± standard deviations (mg/kg FW) of phenolic compounds in hips of *R. pendulina* and the hybrid *R. pendulina* × *spinosissima* in the region of Western Slovenia in 2020. Different letters indicate statistical differences between the two genotypes, separately for flesh with skin and seeds.

Phenolic Group	Compound	*Rosa pendulina* Flesh and Skin	Seeds	*Rosa pendulina* × *spinosissima* Flesh and Skin	Seeds
hydroxybenzoic acid derivatives (HBA)	gallic acid	32.5 ± 2.7 b	4.5 ± 0.5 b	90.8 ± 6.4 a	51.5 ± 5.7 a
	galloyl quinic acid	1055.5 ± 419.4 a	972.0 ± 207.1 a	242.0 ± 17.0 b	137.0 ± 15.4 b
	ellagic acid pentoside 1	53.0 ± 2.1 a	2.1 ± 0.2 a	1.9 ± 0.4 b	0.4 ± 0 b
	ellagic acid pentoside 2	8.0 ± 1.6 b	3.6 ± 0.2 a	1.8 ± 0.1 a	0.8 ± 0.1 b
	TOTAL	1151.0 ± 406.7 a	980.7 ± 210.9 a	336.5 ± 23.9 b	189.7 ± 21.2 b
hydroxycinnamic acid derivatives (HCA)	p-coumaric acid hexoside	0.3 ± 0 b	0.0 ± 0.0 b	20.0 ± 2.5 a	6.3 ± 2.1 a
	5-caffeoylquinic acid 1	5.5 ± 0.7 b	0.84 ± 0.0 b	48.7 ± 2.8 a	17.5 ± 1.7 a
	synapic acid hexoside 1	6.7 ± 0.4 b	0.3 ± 0.03 b	68.4 ± 3.7 a	21.1 ± 3.7 a
	5-caffeoylquinic acid 2	120.7 ± 85.8 b	61.1 ± 3.0 b	768.5 ± 58.0 a	162.0 ± 21.0 a
	5-p-coumaroylquinic acid 1	8.8 ± 1.6 b	2.7 ± 0.2 a	30.0 ± 0.9 a	3.0 ± 0.2 a
	p-coumaric acid hexoside 2	1.1 ± 0.0 b	0.0 ± 0.0 b	2.0 ± 0.2 a	0.6 ± 0.0 a
	5-p-coumaroylquinic acid 2	2.3 ± 0.0 b	0.1 ± 0.0 b	15.0 ± 0.3 a	2.0 ± 0.2 a
	synapic acid hexoside	/	/	24.0 ± 1.8	5.0 ± 0.7
	3-p-coumaroylquinic acid	0.8 ± 0.0 b	0.2 ± 0.0 b	14.0 ± 0.3 a	5.0 ± 0.2 a
	TOTAL	146.2 ± 88.5 b	65.2 ± 3.2 b	990.6 ± 70.5 a	222.5 ± 29.8 a
gallotannins	methyl gallate rutinoside	21.0 ± 0.6 b	3.7 ± 0.9 a	49.4 ± 4.0 a	2.9 ± 0.8 b
	digalloyl hexoside 1	28.6 ± 1.9 b	4.9 ± 1.1 b	70.9 ± 8.6 a	19.0 ± 1.2 a
	methyl gallate hexoside	73.4 ± 0.2 b	22.4 ± 5.7 b	139.6 ± 8.4 a	58.6 ± 4.4 a
	digalloyl hexoside 2	0.7 ± 0.0 b	0.2 ± 0.0 b	19.9 ± 1.9 a	9.6 ± 4.4 a
	digalloyl quinic acid	40.7 ± 4.3 b	9.3 ± 5.7 b	90.3 ± 5.7 a	24.9 ± 7.2 a
	digalloylpentoside	4.7 ± 0.3 b	1.1 ± 0.0 b	17.0 ± 0.5 a	6.5 ± 0.3 a
	TOTAL	169.1 ± 7.3 b	41.6 ± 13.4 b	387.1 ± 29.1 a	121.5 ± 18.3 a
ellagitannins	diHHDP hexoside 1	108.6 ± 8.3 b	31.6 ± 3.3 b	135.4 ± 12.7 a	44.7 ± 4.1 a
	diHHDP hexoside 2	9.7 ± 1.4 b	2.3 ± 0.6 b	29.0 ± 2.0 a	6.0 ± 0.5 a
	HHDP digalloyl hexoside isomer 1	11.0 ± 0.9 b	3.0 ± 0.0 b	43.9 ± 3.8 a	5.9 ± 0.7 a
	HHDP galloylhexoside	29.9 ± 1.9 b	7.1 ± 0.49 b	70.0 ± 3.6 a	6.6 ± 2.1 a
	HHDP digalloyl hexoside isomer 3	14.0 ± 0.2 b	0.7 ± 0.2 b	58.2 ± 6.8 a	11.8 ± 1.0 a
	HHDP digalloyl hexoside isomer 2	8.6 ± 0.1 b	2.7 ± 0.2 b	46.9 ± 10.7 a	11.1 ± 2.7 a
	galloyl bis HHDP hexoside 1	10.5 ± 0.2 a	0.5 ± 0.2 b	4.6 ± 0.6 b	1.4 ± 0.5 a
	galloyl bis HHDP hexoside 2	89.6 ± 4.8 b	14.9 ± 0.7 b	232.8 ± 12.7 a	75.5 ± 0.7 a
	TOTAL	281.9 ± 17.8 b	62.8 ± 5.7 b	620.8 ± 49.3 a	163.0 ± 12.3 a

In terms of the number of phenolic substances identified, the groups of flavanols and flavonols were the most extensive (Table 3). In the group of flavanols, we found catechin, epicatechin, five procyanidin dimers, three procyanidin trimers, PA dimer diglycoside, and two dimer PA monogallates. In general, the contents of various flavanols in the hips of the hybrid *R. pendulina* × *spinosissima* were higher than the contents of flavanols in the hips of *R. pendulina*. The most abundant flavanols in the hips of *R. pendulina* were procyanidin dimer 2 (408 mg/kg FW in the flesh with skin and 110 mg/kg FW in the seeds) and procyanidin dimer 4 (387 mg/kg FW in the flesh with skin and 57 mg/kg FW in the seeds). In the hips of the hybrid *R. pendulina* × *spinosissima*, the flavanol with the highest content was procyanidin dimer 2 (2130 mg/kg FW in the flesh with skin and 93.5 mg/kg FW in the seeds), immediately followed by procyanidin trimer 3, with values of 1490 mg/kg FW in the flesh with skin and 611 mg/kg FW in the seeds. Of the flavonols group, 3 taxifolin pentosides, 12 quercetin derivatives, 1 kaempferol glycoside, and 1 isorhamnetin derivative were determined. The predominant substance in the hips of the *R. pendulina* × *spinosissima* hybrid was quercetingalloyl hexoside 2, with 365 mg/kg FW in the flesh and 170 mg/kg FW in the seeds, while the major flavonol in *R. pendulina* was quercetin-3-glucoside, with 156 mg/kg FW in flesh with skin and 8 mg/kg FW in the seeds (Table 3). Among the flavones, apigenin derivative 1 and apigenin derivative 2 were identified. Their amounts in the rose hips were very low. The results of the study showed that the hybrid *R. pendulina* × *spinosissima* contained significantly higher levels of total flavanols, flavonol glycosides, and flavones. The opposite was true for dihydrochalcones since *R. pendulina* had significantly higher phloridzin content than the hybrid (Table 3).

Table 3. Contents ± standard deviations (mg/kg FW) of phenolic compounds in hips of *R. pendulina* and the hybrid *R. pendulina* × *spinosissima* in the region of Western Slovenia in 2020. Different letters indicate statistical differences between the two genotypes, separately for flesh with skin and seeds.

Phenolic Group	Compound	*Rosa pendulina* Flesh and Skin	Seeds	*Rosa pendulina* × *spinosissima* Flesh and Skin	Seeds
FLAVANOLS	procyanidin dimer 1	102.2 ± 9.2 a	36.8 ± 5.1 b	110.0 ± 60.0 a	50.0 ± 10.0 a
	procyanidin dimer 2	366.4 ± 52.7 b	86.5 ± 26.1 b	1169.7 ± 101.2 a	360.1 ± 40.4 a
	catechin	93.6 ± 5.9 b	22.4 ± 1.5 b	538.6 ± 27.9 a	50.8 ± 16.1 a
	procyanidin trimer 1	104.6 ± 6.6 b	24.9 ± 1.7 a	156.2 ± 8.1 a	14.8 ± 4.7 b
	procyanidin trimer 2	75.4 ± 10.2 b	11.3 ± 2.2 b	343.1 ± 19.8 a	123.2 ± 12.2 a
	procyanidin dimer 3	102.9 ± 11.9 b	6.1 ± 1.2 b	1055.5 ± 6.1 a	416.7 ± 45.8 a
	epicatechin	180.3 ± 9.4 a	23.2 ± 5.8 a	53.9 ± 3.1 b	21.3 ± 2.3 a
	procyanidin dimer 4	387.0 ± 100.0 b	57.0 ± 4.0 b	777.0 ± 370.0 a	162.0 ± 21.3 a
	procyanidin trimer 3	281.7 ± 51.9 b	86.9 ± 6.1 b	768.6 ± 58.0 a	611.0 ± 130.0 a
	procyanidin dimer 5	193.6 ± 3.2 b	9.3 ± 2.9 b	803.3 ± 94.4 a	162.9 ± 13.5 a
	PA dimer diglycoside	121.6 ± 10.5 b	24.6 ± 6.9 b	363.0 ± 130.0 a	354.0 ± 56.0 a
	dimer PA monogallate 1	14.0 ± 4.0 b	2.0 ± 1.0 b	67.6 ± 7.7 a	23.5 ± 3.5 a
	dimer PA monogallate 2	7.0 ± 0.1 b	0.4 ± 0.1 b	29.1 ± 3.4 a	5.9 ± 0.5 a
	TOTAL	1860.3 ± 275.6 b	391.4 ± 64.6 b	6235.6 ± 889.7 a	2356.1 ± 356.3 a
FLAVONOLS	taxifolin pentoside 1	0.8 ± 0.0 b	0.1 ± 0.0 b	6.2 ± 0.9 a	1.1 ± 0.2. a
	quercetin galloyl hexoside 1	1.5 ± 0.0 b	0.9 ± 0.0 a	2.8 ± 0.3 a	0.4 ± 0.0 a
	quercetin-3-rutinoside	7.0 ± 3.6 b	0.4 ± 0.2	0.3 ± 0.0 a	/
	quercetin galloyl hexoside 2	5.0 ± 5.0 b	6.4 ± 0.2 a	8.4 ± 2.5 a	1.1 ± 0.4 b
	quercetin-3-galactoside	7.4 ± 0.5 b	3.8 ± 0.2 b	15.1 ± 0.9 a	6.1 ± 0.4 a
	quercetin-3-glucoside	120.2 ± 7.9 a	7.6 ± 0.2 b	66.1 ± 3.5 b	9.4 ± 0.5 a
	taxifolin pentoside 2	/	/	10.0 ± 0.8	3.0 ± 0.4
	kaempferol hexoside	1.0 ± 0.0 a	0.1 ± 0.0 b	1.0 ± 1.0 a	3.0 ± 0.6 a
	taxifolin pentoside 3	9.0 ± 0.5 b	4.0 ± 0.3 b	30.0 ± 10.0 a	30.0 ± 1.0 a
	phloretin pentosylhexoside	0.6 ± 0.1 b	0.1 ± 0.0 b	1.5 ± 0.3 a	0.9 ± 0.1 a
	quercetin-3-glucuronide	13.0 ± 1.0 b	9.6 ± 1.0 a	66.1 ± 3.5 a	9.4 ± 0.5 a
	quercetin-3-arabinopyranoside	9.9 ± 0.9 b	1.5 ± 0.3 a	14.1 ± 0.7 a	2.0 ± 1.1 a
	quercetin-3-arabinofuranoside	1.9 ± 0.1 b	0.5 ± 0.2 b	4.6 ± 0.4 a	2.9 ± 0.0 a
	isorhamnetin-3-rhamnoside	1.4 ± 0.1 a	0.5 ± 0.0 b	1.7 ± 0.2 a	1.1 ± 0.0 a
	quercetin-3-rhamnoside	10.7 ± 0.8 a	3.3 ± 0.7 a	1.7 ± 0.2 b	1.1 ± 0.0 b
	quercetin-acetylhexoside	1.7 ± 0.1 a	0.5 ± 0.1 a	1.5 ± 0.0 a	0.4 ± 0.1 a
	quercetin galloyl pentoside	0.3 ± 0.0 b	0.2 ± 0.0 b	2.3 ± 0.7 a	2.3 ± 0.9 a
	quercetin-3-xyloside	/	/	7.0 ± 0.9	3.7 ± 0.7
	TOTAL	191.4 ± 20.6 b	39.4 ± 3.2 b	240.4 ± 26.9 a	77.9 ± 6.9 a
FLAVONES	apigenin derivative 1	2.1 ± 1.7 b	0.2 ± 0.0 b	6.8 ± 5.0 a	3.5 ± 0.2 a
	apigenin derivate 2	1.8 ± 0.1 b	1.1 ± 0.0 b	5.5 ± 0.4 a	2.4 ± 0.2 a
	TOTAL	3.9 ± 1.8 b	1.3 ± 0.0 b	12.3 ± 5.4 a	5.9 ± 0.4 a
DIHYDROCHALCONE	phloridzin	2798.8 ± 195.1 a	1467.0 ± 96.6 a	63.4 ± 5.2 b	23.9 ± 6.7 b

The total anthocyanin contents were determined using cyanidin-3-glucoside as a chemical standard. The content was higher in *R. pendulina* × *spinosissima*, 40 mg/kg FW, than in *R. pendulina* hips, 19.3 mg/kg FW (Figure 3).

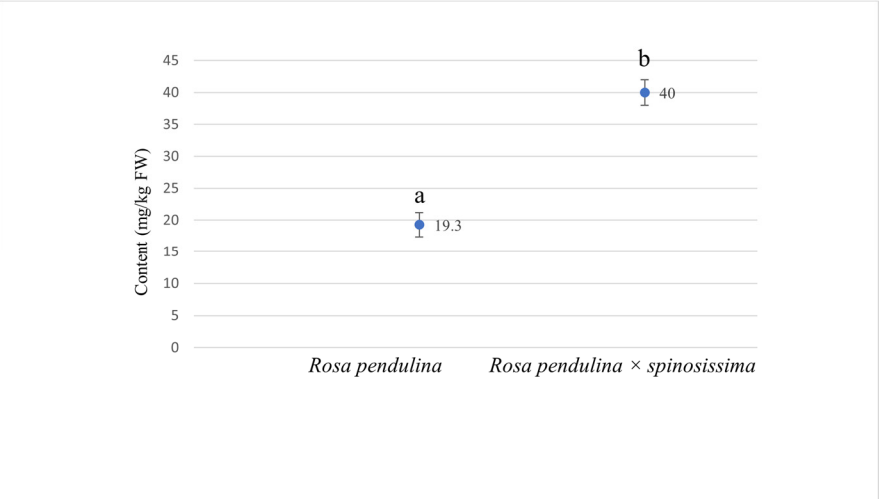


Figure 3. Average contents ± standard deviations (mg/kg FW) of cyanidin-3-glucoside in hips of *R. pendulina* and the hybrid genotype *R. pendulina* × *spinosissima* in the region of Western Slovenia in 2020. Different letters indicate statistical differences between the two genotypes.

4. Discussion

In the present study, the phenolic compounds, organic acids, and vitamin C contents in the fruits of *R. pendulina* and the hybrid with *R. spinosissima* were investigated in detail. Rosehips are generally known to be rich in bioactive compounds such as phenolics, organic acids, and especially vitamin C. However, differences in the contents of these compounds depend on many factors: genetics, the degree of fruit ripeness, growing and storage conditions, extraction, and the method of analysis [18].

The study revealed an unexpectedly large increase in the contents of the analyzed substances in the hybrid involved in the experiment. The content of ascorbic acid in the hybrid, *R. pendulina* × *spinosissima*, was two times higher (1045 mg/100 g FW) than in the main species, *R. pendulina* (535 mg/100 g FW). A comparison of the results of our study with the results of other studies shows that other authors have also reported that hips are one of the richest sources of vitamin C. Our results confirm that hips are a rich source of vitamin C. Our measured values for vitamin C are in accordance with the literature for *Rosa* spp. [19–21], but the value for *R. pendulina* × *spinosissima* stood out. Bayramoğlu et al. [19] reported a vitamin C content of 430.69 µg/100 g in *R. pisiformis* (H.Christ) Sosn. Javanmard et al. [20] analyzed the vitamin C contents in five different ecotypes of *R. canina*. The contents ranged from 50 mg/100 g FW to 1360 mg/100 g FW. Nybom and Werlemark [22] further reported that, in general, local environmental conditions strongly influence rosehip yield and quality. A colder climate probably results in higher vitamin C contents. This would also have affected our results since the rose hips were harvested at higher altitudes.

The hybrid in our experiment also had higher contents of organic acids. The determined acids were citric, malic, quinic, shikimic, and fumaric. Quinic acid was the major organic acid in the hybrid, *R. pendulina* × *spinosissima* (48.8 g/kg FW), while the content of this acid was only 0.29 g/kg FW in the original species, *R. pendulina*. Kerasioti et al. [23] reported that the quinic acid contents in their samples ranged from 389.9 to 1102.6 µg/g DW. Surprisingly, no malic acid was present in the hips of the hybrid, although its content was 1.3 g/kg FW in the main species. Malic acid was the most represented acid in the main species [23]. Citric acid was expected to be the most abundant in both genotypes, but it was not. Small quantities of shikimic and fumaric acid were present in both geno-

types. Cunja et al. [24] also analyzed the citric acid content in *R. canina* and found it to be 15–18 mg/100 g DW. Okatan et al. [25] reported that the citric acid contents in their samples ranged from 15.9 to 22 g/kg FW. Our content in *R. pendulina* was lower than the reported content, but it should be noted that research on other roses was not performed in the same way as our research. Bozan et al. [26] reported that the content of citric acid in *R. canina* hips ranged from 5.9 g/100 g FW to 7.5 g/100 g FW. In our study, it was found only in *R. pendulina*, with a content 1.3 g/kg FW. If the content of citric acid is compared with the results from Okatan et al. [25], our citric acid content was much lower. Okatan et al. [25] reported a content between 1.2 and 3.2 g/100 g FW. Fumaric and shikimic acid were present in our research in low amounts. Slightly higher values were reported by Cunja et al. [24].

The contents of gallotannins, ellagitannins, flavanols, flavonols, flavones, and hydroxycinnamic acids and their derivates were higher in the hybrid *R. pendulina* × *spinosissima*, except for the contents of hydroxybenzoic acid derivatives (HBA) and phloridzin, which were higher in *R. pendulina*. The contents of flavanols in the original species were 1860.3 mg/kg FW in flesh with skin and 391.4 mg/kg FW in seeds. In the hybrid, these values were 6235.6 mg/kg FW in the flesh with skin and 2336.1 mg/kg FW in the seeds. The contents of flavonols were very low in the parent species, 191.4 mg/kg FW in the flesh with skin and 39.4 mg/kg in the seeds. The contents in hips of the hybrid genotype were significantly higher, being 240.4 mg/kg in the flesh with skin and 77.9 mg/kg in seeds. Surprisingly, phloridzin was the prevailing component in *R. pendulina*. We believe that the significant differences in chemical composition between the two studied Rosa types are due to genotype. Roman et al. [3] also concluded that differences in the chemical constitution of eight genotypes of *R. canina* from Transylvania were due to genetic variations as well as growing altitudes. All plants were harvested during the same period of the year, from September to October, but were grown under different ecological conditions. It is known that plant genotype, cultivation site, and technique affect the total phenolic content in hips [3]. Javanmard et al. [20] analyzed the total phenolic content of *R. canina* hips, and their results varied from 73.39 mg GAE/g DW to 104.7 mg GAE/g DW. The total analyzed phenolic content in rose hips was higher in *R. pendulina* × *spinosissima* flesh and seeds than in *R. pendulina*. Cunja et al. [24] also analyzed the total phenolic content in *R. canina* hips at six different times of harvest. The total phenolic content ranged from 12.3 to 15.7 mg ekv GAE/g DW. Our results show that seeds were poorer in total phenolic content than flesh. Only one anthocyanin was identified in the hips, cyanidin-3-glucoside, at values from 0.92 to 13 mg/kg DW [24]. We also determined only cyanidin-3-glucoside in the hips. Its content was higher in *R. pendulina* × *spinosissima* (40.0 mg/kg FW) than in *R. pendulina* (19.3 mg/kg FW). Anthocyanins were not determined in seeds.

Based on the findings of our research and the hypotheses put forward, we can confirm that a heterosis effect is present in our analyzed hybrid, which results in the hybrid having a higher concentration of bioactive compounds. Hybridization is a very common phenomenon in the genus *Rosa*. However, very little is known about the influence of the hybridization process on the contents of various bioactive compounds in rose hips. Knowledge of the influence of the hybridization process on the distribution of various substances in the rosehip is insufficient. Gustafsson [27] previously reported that the ascorbic acid content of *R. canina* was much higher in hybrid plants than in the parent plant but lower than in the paternal plant. This is in direct agreement with our results. The *R. pendulina* × *spinosissima* hybrid has a higher ascorbic acid content, a higher total phenolic content, and a higher content of cyanidin-3-glucoside, as the main rosehip anthocyanin, than the original *R. pendulina* mother plant. Forkmann [28] reported that a single gene may be responsible for differences in the contents of different anthocyanins. We assume that the dominant and recessive alleles of this gene, which is responsible for the synthesis of phenols, were probably transferred to our hybrid and formed a heterozygous form, and the effect of heterosis was therefore present. The effect of heterosis was also the driving process in the case of differences in other substances.

In addition, based on the obtained results, we can confirm the hypothesis that the content of phenolic compounds in the flesh with the skin of all analyzed rose hips is higher compared to the content in the seeds.

5. Conclusions

It can be concluded that the hips of the hybrid *R. pendulina* × *spinosissima*, collected in 2020 in Western Slovenia, have a higher content of bioactive compounds than the original parental species *R. pendulina*. The increase in compound levels is based on heterosis. As expected, the contents of secondary metabolites in the seeds were much lower than in the flesh with the skin. Our results show that the studied roses, especially the hybrid genotype, are a rich source of secondary metabolites, organic acids, and vitamin C. The content of vitamin C in rose hips is very high, so it can be said that rose hips are an excellent source of vitamin C for strengthening the human immune system. The findings contribute to a better understanding of changes in the contents of different bioactive compounds in rose hips due to crossing. The results will contribute to greater transparency of the knowledge of which types of roses produce rose hips that are best suited for nutritional purposes. Additionally, further research on the natural hybrids of the genus *Rosa* and whether the contents and compositions of the bioactive substances they contain are suitable for human consumption will be encouraged.

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Appendix A

Table A1. Details of all chemical standards used (supplier and purity) and standards used for the calculation of contents in rose hips.

Chemical	Supplier	Purity	Calibration Curve Equation (mg/mL)	R2
organic acids				
citric acid	Sigma-Aldrich	≥99.5	35.196	0.9998
malic acid	Sigma-Aldrich	≥97%	23.124	0.9982
quinic acid	Supelco	≥98%	11.868	0.9933
shikimic acid	BioChemika	≥97%	1666.3	0.9994
fumaric acid	Sigma-Aldrich	≥99%	3860.3	0.9967
ascorbic acid	Sigma-Aldrich	≥99%	1964.50	0.9974
phenolic compounds				
cyanidin-3-glucoside	Sigma-Aldrich	≥95%	384.06	0.9993
kaempferol-3-glucoside	Sigma-Aldrich	≥98%	970.75	1.00
isorhamnetin-3-glucoside	Extrasynthese	≥98%	529.29	1.00
quercetin-3-rutinoside	Sigma-Aldrich	≥95%	122.28	0.98

Table A1. *Cont.*

Chemical	Supplier	Purity	Calibration Curve Equation (mg/mL)	R2
quercetin-3-galactoside	Sigma-Aldrich	≥97%	291.06	1.00
quercetin-3-xyloside	Apin Chemicals	≥98%	972.86	1.00
quercetin-3-rhamnoside	Sigma-Aldrich	≥95%	645.63	0.97
quercetin-3-arabinofuranoside	Apin Chemicals	≥98%	695.8	0.9991
quercetin-3-glucoside	Sigma-Aldrich	≥97%	349.36	0.9998
quercetin-3-arabinopyranoside	Apin Chemicals	≥98%	356.97	0.996
caffeic acid	Sigma-Aldrich	≥98%	1656.8	1.00
apigenin-7-glucoside	Sigma-Aldrich	≥97%	549.68	0.9979
3-caffeoylquinic acid	Sigma-Aldrich	≥98%	1656.8	1.00
4-caffeoylquinic acid	Sigma-Aldrich	≥98%	1656.8	1.00
chlorogenic acid	Sigma-Aldrich	≥95%	466.48	1.00
epicatechin	Sigma-Aldrich	≥97%	191.97	0.9963
catechin	Supelco	≥99%	116.26	0.9985
p-coumaric acid	Sigma-Aldrich	≥98%	1742.52	1.00
procyanidin B1	Supelco	≥90%	74.34	1.00
ellagic acid	Sigma-Aldrich	≥95%	150.06	0.99

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