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Recommended Citation

Bhave, A.; Schulzova, V.; Chmelarova, H.; Mrnka, L.; and Hajslova, J. (2017) "Assessment of rosehips based on the content of their biologically active compounds," *Journal of Food and Drug Analysis*: Vol. 25 : Iss. 3 , Article 8. Available at: <https://doi.org/10.1016/j.jfda.2016.12.019>

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Original Article

Assessment of rosehips based on the content of their biologically active compounds



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ARTICLE INFO

Article history:

Received 20 October 2016

Received in revised form

13 December 2016

Accepted 25 December 2016

Available online 17 February 2017

Keywords:

antioxidant activity

carotenoids

flavonoids

tocopherols

triterpenic acids

ABSTRACT

In this study, an in-depth analysis of the unique set of rosehip samples from 71 *Rosa* genotypes was conducted with the aim to identify the most suitable ones for applications in the food and pharmaceutical industries based on the content of biologically active compounds. In the first part of our experiments, the antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl assay and the genotypes with the highest values were selected for the follow-up analysis. In the second part of experiments, the major classes of biologically active compounds in rosehips such as carotenoids, tocopherols, flavonoids, and triterpenic acids were further quantified using liquid chromatography-based techniques. Large variation was observed among all the analyzed compounds with intraspecific variation often hiding interspecific or inter-sectional differences. The compounds studied herein thus do not provide a sharp tool for chemotaxonomic resolution of the genus *Rosa*. High intraspecific variation indicates the necessity to screen and utilize individual rose genotypes rather than representatives of the species when searching for sources of biologically active compounds. In the final stage of the study, 10 genotypes were selected for further cultivation and use, based on the highest concentrations of the analyzed biologically active compounds.

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1. Introduction

Consumers' growing interest in herbal food supplements and nutraceuticals has accelerated the search for raw materials rich in biologically active compounds. Rosehips are the aggregate fruits of shrubs belonging to the *Rosa* genus of the Rosaceae family that are widely used by both food and

pharmaceutical industries. The genus comprises nearly 200 species with complex taxonomy [1,2]. Roses are widespread in temperate to subtropical habitats of Europe, Asia, Middle East, and North America [3,4]. Rosehips are found in varied sizes and colors from yellow-orange to dark red and sometimes even black, depending on the pattern of pigments such as carotenoids, flavonoids, or anthocyanins.

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<http://dx.doi.org/10.1016/j.jfda.2016.12.019>

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Rosehips contain a large range of important dietary antioxidants. The high antioxidant activity is mainly attributed to ascorbic acid that typically ranges from 3 g/kg to 40 g/kg [5], which is fairly more than any other commonly available fruits or vegetables [6]. Apart from ascorbic acid, carotenoids represent mainly lycopene, β -carotene, and only traces of lutein and zeaxanthin [7]. Tocopherols detected in rosehips include α - and γ -tocopherols [8,9], and polyphenolic compounds include flavonoids and proanthocyanidins [10,11]. In rosehips, flavonoids comprise glycoside derivatives of quercetin, including quercitrin (quercetin-3-O-rhamnoside), isoquercitrin (quercetin-3-O-glucoside), and hyperoside (quercetin-3-O-galactoside) [10,12–14], and some aglycones, including catechin, quercetin, taxifolin, and eriodictyol [10]. Triterpenic acids present in rosehips are primarily known for their immunomodulatory properties [15]. Ursolic and oleanolic acids have shown hepatoprotective, anti-inflammatory, antitumor, and antihyperlipidemic effects in *in vitro* and *in vivo* experiments [15–18], while betulinic acid is well known for other biological activities such as inhibition of human immunodeficiency virus and antibacterial, antimalarial, anti-inflammatory, anthelmintic, and antioxidant properties [15,19]. Other significant groups of biologically active compounds found in rosehips are galactolipids with their anti-inflammatory, antioxidant [20], antiviral, and antitumor activities [21]. Unsaturated fatty acids found in rosehip seeds, mainly linoleic and α -linolenic acids, have been considered responsible for the inhibitory effects on cyclooxygenase 1 and 2 in *in vitro* experiments [22].

Rosehips being rich sources of biologically active compounds, analytical studies were conducted to explore the health-promoting compounds, which focused mainly on samples from a particular region or were restricted to a particular variety/species with limited chemical analysis [4,23–26]. The increasing importance of rosehips as food supplements triggered the need to analyze and find the best species/genotype for the future. The novelty of this study was to critically assess the unique set of 71 rose genotypes that were all grown in the same conditions (to erase the environmental effects), and observe the influence of various genotypes and sections with regard to the content of health-promoting compounds occurring in rosehips. Previously, no study was carried out in such detail to compare and analysis the biologically active compounds occurring in them. For this, the total antioxidant activity was determined, followed by an analysis of selected biologically active compounds using liquid chromatography (LC)-based techniques. In the end, best rose genotypes were selected based on the highest content of biologically active compounds for future agricultural purposes and later use in commercial applications of rosehips.

2. Methods

2.1. Chemicals and reagents

Methanol [high-performance liquid chromatography (HPLC) grade], n-hexane (chromatography grade), and ethanol ($\geq 99.5\%$) were purchased from Merck (Darmstadt, Germany).

Formic acid ($\sim 98\%$), ethyl acetate, and Pestanal were purchased from Fluka Analytical (Steinheim, Germany). Deionized water was prepared with a Milli-Q purification system from Millipore (Eschborn, Germany). All the other chemicals such as ammonium formate ($\geq 99.0\%$), acetonitrile, Chromasolv (HPLC grade, $\geq 99.9\%$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and *tert*-butyl-hydroxytoluene (*t*-BHT) were purchased from Sigma-Aldrich (Steinheim, Germany). Analytical standards used as references, including *L*-ascorbic acid ($\geq 99\%$), β -carotene ($\geq 97\%$), lycopene ($\geq 90\%$), α -tocopherol ($\geq 95.5\%$), γ -tocopherol ($\geq 95.5\%$), δ -tocopherol ($\geq 90\%$), quercetin ($\geq 95\%$), catechin ($\geq 98\%$), rutin ($\geq 95\%$), betulinic acid ($\geq 98\%$), oleanolic acid ($\geq 97\%$), and ursolic acid ($\geq 90\%$), were also purchased from Sigma-Aldrich.

2.2. Sample material

Rosehips belonging to 71 different genotypes (both pure species and hybrids), coming from the rose collection of the Institute of Botany, Academy of Sciences of the Czech Republic (49°59'34.994"N, 14°34'8.266"E, Průhonice, Czech Republic), were used for the analysis. The selected genotypes belonged to seven rose sections: Bracteatae, Caninae, Carolinae, Cinnamomeae, Pimpinellifoliae, Rosa, and Synstylae (Table 1). If not stated otherwise, nomenclature and section affiliation followed that of Bruneau et al [1], and Wissemann and Ritz [2]. The ripened rosehips were harvested in the beginning of October 2012 before the drop of the minimum temperature below 0°C. Fruits were dried to a constant weight at 35°C (7–10 days) and then stored at room temperature prior to analysis. Prior to extraction, samples were deseeded manually by breaking the hips and further crushed in a mortar and pestle to a fine size.

A unique set of 71 rosehip samples from different genotypes was organized systematically for the analysis of their biologically active compounds. Primarily, antioxidant activity (DPPH assay) was determined in all samples.

2.3. DPPH radical scavenging activity

Each sample was extracted by shaking 0.5 g crushed rosehip shell with 40 mL deionized water (Milli-Q purification system; Millipore) for 1 hour on a rotary shaker. For the determination of the antioxidant activity, 2 mL of methanolic solution containing DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals (5.2 mg/L) was added to 1 mL of the prepared extracts, reference standards, and control samples. A linear calibration plot was plotted using freshly prepared aqueous solution of *L*-ascorbic acid, with concentration ranging from 1.08 mg/L to 6.45 mg/L, and deionized water was used as a blank. The reaction mixture was kept in dark for 60 minutes before the measurement, and the reduction of DPPH free radicals was measured at 517 nm wavelength using the Spectrophotometer Cary 100 Bio (Agilent Technologies, Palo Alto, CA, USA). Linear equations from reference standards were used to calculate the concentration of antioxidants, expressed as ascorbic acid equivalents. The method was validated using linear equation, and the relative standard deviation (RSD) was obtained to be 3.6%.

Table 1 – Taxonomical overview of the rose genotypes examined in this study.

Section	Specimen ^a	
	No.	Taxonomic identity (genotype code)
Bracteatae	1	<i>Rosa bracteata</i> (8/7)
Caninae	30	<i>Rosa agrestis</i> (9/2), <i>R. agrestis</i> or <i>Rosa micrantha</i> (D9/1), <i>Rosa andegavensis</i> (12/10)*, <i>Rosa caesia</i> (15/1), <i>R. canina</i> (1/6), <i>R. canina</i> (6/7), <i>R. canina</i> × <i>Rosa majalis</i> (7/10), <i>Rosa corymbifera</i> (4/10), <i>R. corymbifera</i> × <i>R. andegavensis</i> (2/5), <i>R. dumalis</i> (3/6), <i>R. dumalis</i> (L4/2), <i>R. dumalis</i> (3/5)*, <i>R. dumalis</i> (D5/3), <i>R. dumalis</i> (DX/1), <i>R. dumalis</i> (L9/1)*, <i>R. dumalis</i> (4/1), <i>R. dumalis</i> (L4/1)*, <i>Rosa inodora</i> (L13/1), <i>R. inodora</i> (L13/2), <i>Rosa jundzillii</i> (5/2), <i>R. micrantha</i> (15/3), <i>R. pulverulenta</i> (1/7), <i>R. pulverulenta</i> (10/10)*, <i>R. rubiginosa</i> (4/7), <i>R. rubiginosa</i> (14/8)*, <i>Rosa serafinii</i> (16/3), <i>R. sherardii</i> or <i>R. villosa</i> (L8/2)*, <i>Rosa subcanina</i> (2/7), <i>R. subcanina</i> × <i>R. dumalis</i> (9/8)*, <i>R. villosa</i> (1/3)
Carolinae	1	<i>Rosa virginiana</i> (L8/1)
Cinnamomeae	10	<i>Rosa blanda</i> (2/6), <i>Rosa fedtschenkoana</i> (L2/1), <i>R. majalis</i> (3/4), <i>R. majalis</i> (14/2), <i>Rosa multibracteata</i> (9/4), <i>R. prattii</i> (15/10)*, <i>Rosa hemsleyana</i> (4/9)*, <i>Rosa sweginzowii</i> (15/6), <i>Rosa woodsii</i> (1/4), <i>R. woodsii</i> (L7/2)
Pimpinellifoliae	3	<i>Rosa elasmacantha</i> (2/3)*, <i>R. spinosissima</i> (6/2)*, <i>R. spinosissima</i> (2/10)
Rosa	3	<i>R. alba</i> (15/9)*, <i>Rosa gallica</i> (6/1), <i>R. gallica</i> (D6/1)
Synstylae	6	<i>R. arvensis</i> (1/2), <i>R. arvensis</i> (L18/1), <i>Rosa filipes</i> (12/3), <i>R. henryi</i> (12/6), <i>R. multiflora</i> (1/8), <i>R. multiflora</i> (1/9)
Caninae × Cinnamomeae	9	<i>R. caesia</i> × <i>R. pendulina</i> (7/4), <i>R. corymbifera</i> × <i>R. majalis</i> (15/8)*, <i>Rosa glauca</i> × <i>R. majalis</i> (5/4)*, <i>R. inodora</i> × <i>R. pendulina</i> (7/1)*, <i>R. inodora</i> × <i>R. pendulina</i> (L7/1), <i>R. rubiginosa</i> × <i>Rosa rugosa</i> (D5/2), <i>R. rugosa</i> × <i>R. villosa</i> (D5/1), <i>Rosa tomentella</i> × <i>R. majalis</i> (6/3), <i>R. villosa</i> × <i>Rosa laxa</i> (3/10)
Caninae × Rosa	2	<i>Rosa zalana</i> (12/7)*, <i>R. zalana</i> × <i>villosa</i> (5/5)
Caninae × Synstylae	1	<i>R. rubiginosa</i> × <i>R. arvensis</i> (5/1)
Cinnamomeae × Pimpinellifoliae	3	<i>R. majalis</i> × <i>R. spinosissima</i> (D6/2), <i>R. spinosissima</i> × <i>R. reversa</i> (4/4)*, <i>R. reversa</i> (9/3)
Synstylae × Rosa	1	<i>R. arvensis</i> × <i>R. gallica</i> (16/8)
Caninae × Cinnamomeae × Rosa	1	<i>R. zalana</i> × <i>R. rugosa</i> (L7/3)

^a Samples typed in bold font were selected for carotenoid, tocopherol, and flavonoid analyses and those marked by “*” were analyzed for triterpenic acids.

2.4. Rosehip component analysis

For the determination of carotenoids, tocopherols, and flavonoids, 33 samples with high and three with low antioxidant activity were selected for comparison. The selected samples are typed in bold font in Table 1.

2.4.1. Carotenoids and tocopherols

The samples (1 g) were extracted by a mixture of 4 mL of ethanol with 0.2% (*w/v*) of *t*-BHT used as an antioxidant and 4 mL of hexane. The suspension was shaken well before adding 1 mL of water, followed by further 30 minutes of shaking and centrifugation at 10,000 *g* for 5 minutes. The supernatant organic layer was collected in the evaporating flask. Addition of hexane (4 mL) along with the shaking–centrifugation step was repeated three more times, and the supernatant organic layer from each extraction was combined and evaporated to dryness on a rotary shaker. The residue was reconstituted in 2 mL of ethanol (with 0.2% *t*-BHT):acetone [6:4 (*v/v*)] and microfiltered by 0.2 μ m PVDF (polyvinylidene fluoride) membrane filter. The calibration range was from 0.1 μ g/mL to 20 μ g/mL, prepared in the solvent mixture of ethanol (with 0.2% *t*-BHT):acetone [6:4 (*v/v*)] for carotenoids (β -carotene and lycopene) along with tocopherols (α -, γ -, and δ -tocopherols).

2.4.1.1. Instrumental setup. Determination of carotenoids and tocopherols was performed simultaneously using the LC

system 1200 series (Agilent Technologies) and Kinetex C18 analytical column (100 × 2.1 mm² i.d., 2.6 μ m; Phenomenex, Aschaffenburg, Germany) held at a constant column temperature of 30°C. The mobile phases were (A) water and (B) methanol with gradient elution as follows: 0–2 minutes of isocratic elution at 90% of B, 2–5 minutes of linear gradient elution from 90% to 100% of solvent B, 5–28 minutes of isocratic elution at 100% of solvent B, 28–28.5 minutes of linear elution from 100% to 90% of solvent B, and finally, 28.50–30 minutes of isocratic elution at 90% of solvent B with a flow rate 0.5 mL/min. The employed detectors included diode array detector (DAD) and fluorescence light detector (FLD). DAD measurements were recorded at wavelength 470 nm for lycopene and 450 nm for β -carotene in parallel to FLD at 290 nm excitation and 330 nm emission wavelengths for tocopherols. The method was validated using linear equation, and the RSD values obtained were 2.7% for carotenoids and 3.8% for tocopherols.

2.4.2. Polyphenolic compounds/flavonoids

The samples (0.5 g) were extracted by shaking with 30 mL of acidified methanol [5% (*v/v*)] and formic acid for 1 hour, followed by centrifugation at 10,000 *g* for 5 minutes, filtration using 0.2 μ m PVDF membrane filter, and finally diluted with methanol prior to analysis. The calibration curve was obtained using reference of quercetin, catechin, and rutin prepared in methanol, varying in concentration from 10 ng/mL to 1000 ng/mL.

2.4.2.1. Instrumental setup. Detection and semiquantification of flavonoids were performed using an ultra-high-performance liquid chromatography–high-resolution tandem mass spectrometry (UHPLC–HRMS/MS) system consisting of UltiMate 3000 RSLC (Thermo Fisher Scientific, Waltham, MA, USA) coupled with the QExactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The analytical column used for separation was Kinetex C18 ($100 \times 2.1 \text{ mm}^2$ i.d., $1.7 \mu\text{m}$; Phenomenex) with column temperature set at 40°C . The mobile phases were as follows: acidified water [0.1% formic acid in water (v/v)] (A) and acidified methanol [0.1% formic acid in methanol (v/v)] (B) with gradient elution as follows: 0–8 minutes of linear gradient elution from 5% to 100% of solvent B, 8–13.5 minutes of isocratic elution at 100% of solvent B, 13.5–13.6 minutes of linear elution from 100% to 5% of solvent B, and 13.6–15 minutes of isocratic elution at 5% of solvent B at a flow rate of 0.4 mL/min. The HRMS/MS detection took place using electrospray ionization operated simultaneously in positive and negative ionization modes in the scan range from 100 m/z to 1200 m/z . Identification of unknown compounds was performed in the negative ion mode because of better sensitivity, based on their retention time, exact m/z values, elemental composition generated by the Xcalibur software (version 2.1, Thermo Fisher Scientific, Waltham, MA, USA), and mass spectra, and fragments obtained for a particular ion in full MS–data-dependent MS/MS mode and full MS–product reaction monitoring MS/MS mode. The lists of polyphenolic compounds found in rosehips with their characteristic fragments are shown in Table 2. Semiquantification of these compounds was conducted using rutin as the reference standard because of a similar estimated response of detected flavonoid glycosides. The method was validated, and the RSD for the reference standard was obtained to be 5.3%.

2.4.3. Triterpenoic acids

The samples (1 g) were extracted by shaking with 30 mL ethyl acetate followed by centrifugation at 10,000 g for 5 minutes. From this extract, 2 mL aliquot was evaporated in an

evaporating flask and reconstituted with 10 mL of methanol. The reconstituted extract was filtered using $0.2 \mu\text{m}$ PVDF membrane filter and diluted with methanol prior the HPLC–HRMS analysis. The calibration curve was obtained using betulinic, oleanolic, and ursolic acids in methanol, varying in concentration from 10 ng/mL to 1000 ng/mL.

2.4.3.1. Instrumental setup. Triterpenoic acids were detected and quantified using an HPLC–HRMS system consisting of Acquity LC (Waters, Milford, MA, USA) coupled with the Orbitrap mass spectrometer Exactive (Thermo Fisher Scientific, Bremen, Germany). The analytical column used was LC-Supelcosil ($250 \times 4.6 \text{ mm}^2$ i.d., $5 \mu\text{m}$; Sigma-Aldrich) with the constant column temperature set at 40°C . The mobile phases were 10 mM ammonium formate (w/v) with formic acid at (A) pH 3 and (B) acetonitrile with gradient elution as follows: 0–3 minutes of isocratic elution at 85% of solvent B, 3–15 minutes of linear gradient elution at 100% of solvent B, 15–17 minutes of isocratic elution at 100% of solvent B, and 17–18.5 minutes of isocratic elution at 85% of solvent B, at a flow rate 0.7 mL/min. The HRMS detection took place with an atmospheric pressure chemical ionization ionization source operated simultaneously in positive and negative ionization modes in the scan range from 50 m/z to 1200 m/z . This LC method was adopted from Wenzig et al's [15] study and modified. Concentrations of triterpenoic acids in rosehips were evaluated in the negative detection mode since it provided better sensitivity. The method was validated and the RSD for reference standard was obtained to be 6.2%.

2.5. Chemometric analysis

Statistical evaluation of the acquired data was performed using commercial software SIMCA (version 13.0.0.0, 2012; Umetrics, Malmo, Sweden). Prior to chemometric analysis, the data were normalized by the constant row sum method. Following this, the data were processed for unsupervised principal component analysis (PCA) and supervised partial least square discriminant analysis (PLS-DA) models, to assess

Table 2 – Polyphenolic compounds found in rose hip extracts with their characteristic fragments in the negative ion mode.

Identified compounds	Formula	Theoretical m/z [M–H]–	Acquired m/z [M–H]–	Difference m/z values (ppm)	Retention Time (min)	Fragments (m/z value)
Catechin ^a	$\text{C}_{15}\text{H}_{14}\text{O}_6$	289.0712	289.0721	4.7	2.32	245.0817
Quercetin ^a	$\text{C}_{15}\text{H}_{10}\text{O}_7$	301.0348	301.0349	2.1	4.13	151.0036, 178.9988
Methyl gallate hexoside	$\text{C}_{14}\text{H}_{18}\text{O}_{10}$	345.0822	345.0827	3.03	2.17	183.0298
Quercetin pentoside	$\text{C}_{20}\text{H}_{18}\text{O}_{11}$	433.0771	433.0771		4.37	300.0274, 301.0350
Taxifolin pentoside (1)	$\text{C}_{20}\text{H}_{20}\text{O}_{11}$	435.0927	435.0929	1.7	3.48	285.0407, 151.0037
Taxifolin pentoside (2)	$\text{C}_{20}\text{H}_{20}\text{O}_{11}$	435.0927	435.0887	–7.9	3.95	285.0406, 151.0036
Phloridzin	$\text{C}_{21}\text{H}_{24}\text{O}_{10}$	435.1291				
Quercitrin	$\text{C}_{21}\text{H}_{20}\text{O}_{11}$	447.0927	447.0933	2.39	4.39	300.0271, 301.0355
Eriodictyol hexoside (1)	$\text{C}_{21}\text{H}_{22}\text{O}_{11}$	449.1084	449.1082	0.91	2.9	259.0613, 269.0457
Eriodictyol hexoside (2)	$\text{C}_{21}\text{H}_{22}\text{O}_{11}$	449.1084			3.5	287.0564, 151.0036
Eriodictyol hexoside (3)	$\text{C}_{21}\text{H}_{22}\text{O}_{11}$	449.1084	449.1089	2.34	3.67	269.0456, 151.0036
Catechin hexoside	$\text{C}_{21}\text{H}_{24}\text{O}_{11}$	451.124	451.1249	3.08	3.79	341.0670, 217.0145
Isoquercitrin	$\text{C}_{21}\text{H}_{20}\text{O}_{12}$	463.0877	463.0886	3.14	4.15	300.0277, 301.0356
Quercetin hexuronide	$\text{C}_{21}\text{H}_{18}\text{O}_{13}$	477.0669	477.0671	1.55	4.11	301.0356, 314.0437
Rutin ^a	$\text{C}_{27}\text{H}_{30}\text{O}_{16}$	609.1456	609.1463	2.11	4.13	300.0277, 301.0360

^a Identification confirmed by standards.

the observed variability in particular groups of compounds and to test the chemometric separation of the sections/species. Pearson's correlation coefficient was also determined for antioxidant activity and separately analyzed group of compounds (carotenoids, tocopherols, flavonoids, and triterpenic acids).

3. Results and discussion

Similar to other medicinal plants, not only genotype, but also a wide range of environmental factors (e.g., climate, soil, difference in altitude of agricultural land, etc.) may impact the rosehip composition [23,24,26]. In this study, we explored the unique set of 71 samples that represented various rose genotypes grown in the same locality, thus minimizing the impact of environmental factors. To identify the genotypes most suitable for large-scale cultivation, a comprehensive evaluation of their compositional characteristics, related to the quality criteria required by food supplement and “healthy food” producers, was performed.

3.1. DPPH radical scavenging activity

Antioxidant activity was considered as a key criterion due to the presence of potent antioxidants in rosehips and their importance for human health. For this purpose, a spectrophotometric method was employed based on the DPPH radical scavenging activity. Values of antioxidant activity widely ranged from 10 g/kg up to 349 g/kg of ascorbic acid equivalent for dried rosehips in *Rosa multiflora* (1/9) and *Rosa prattii* (15/10). The activity measured in this study was expressed as ascorbic acid equivalent because this compound is a major antioxidant compound occurring in rosehips [13]. Besides DPPH, other scavenging activities such as ferric-reducing antioxidant power were used in literature to study the antioxidant activity of rosehips. For instance, Gao et al [13] analyzed the antioxidant activity using the ferric-reducing antioxidant power method in the genotypes *Rosa canina* and *Rosa villosa*, and found that the activity ranged from 86.5 g/kg to 192.5 g/kg of

ascorbic acid equivalent for dried rosehips. It is worth noting that, although widely used, these methods measure the total antioxidant activity and do not enable differentiation between antioxidant compounds present in the sample. We hypothesized that in rosehips not only ascorbic acid, but also phenolics, terpenoids, or carotenoids are responsible for the antioxidant activity, and hence we analyzed the content of these compounds. The difficulty to correlate radical scavenging activity with a single component was thoroughly discussed by Ghazghazi et al [4] and was also confirmed by our results, as seen in section Chemometric analysis.

In order to learn more on the biologically active compounds participating in the antioxidant activity of rosehips, an in-depth analysis was carried out using LC-based techniques.

3.2. Rosehip component analysis

Follow-up experiments were carried out to analyze the levels of biologically active compounds other than ascorbic acid, which are known for their therapeutic properties. For experimental purpose, the samples were divided into categories of high and low antioxidant activities considering 135 g/kg of ascorbic acid equivalent for dried rosehips as the critical value. D of carotenoids, tocopherols, and flavonoids was conducted for 33 samples with highest activity, and three samples with low activity were selected for comparison (Table 1). The results obtained are summarized in the three subsequent sections for carotenoids, tocopherols, and flavonoids.

3.2.1. Carotenoids

Determination of carotenoids was carried out using HPLC–DAD. All the chromatograms were screened individually for the peaks with carotenoid spectra. The chromatographic separation of carotenoids occurring in rosehips can be found in Figure S1(a). Significant intensity was obtained for β -carotene and lycopene peaks in all analyzed rosehip samples, and hence these carotenoids were quantified using reference standards. The individual contents of the targeted carotenoids in all analyzed samples were in the range from a value lower

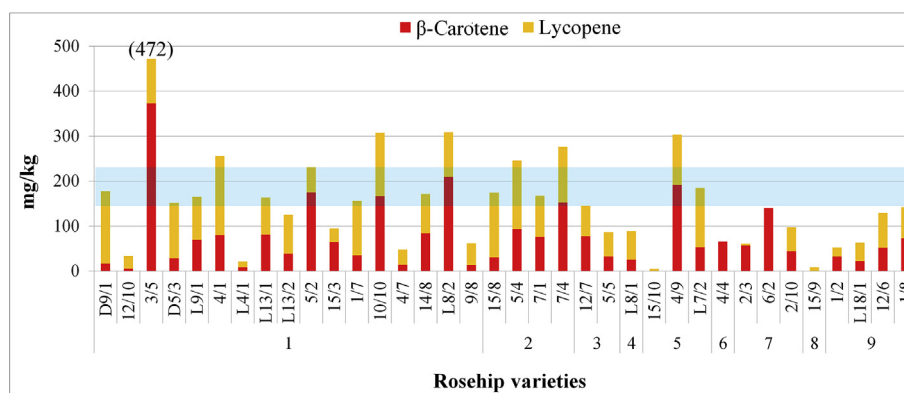


Figure 1 – Graphical representation of carotenoids in the fruits of rose genotypes. The carotenoid content is categorized based on the percentiles (mean value being 149 mg/kg): low (percentiles 0–25), moderate (percentiles 26–75) as marked in blue; and high (percentiles 76–100). Numerical labeling for rosehip sections are as follows: 1, *Caninae*; 2, *Caninae* × *Cinnamomeae*; 3, *Caninae* × *Rosa*; 4, *Carolinae*; 5, *Cinnamomeae*; 6, *Cinnamomeae* × *Pimpinellifoliae*; 7, *Pimpinellifoliae*; 8, *Rosa*; and 9, *Synstylae*.

than the limit of detection (LOD; ~ 0.16) to 373 mg/kg for β -carotene and from a value lower than the LOD (~ 0.16) to 176 mg/kg for lycopene (Figure 1). Our sample set also included rosehip genotypes *Rosa dumalis* (3/5 and D5/3), *R. dumalis* hybrid (9/8, L9/1, 4/1, and L4/1), *Rosa rubiginosa* (4/7, 14/8, D5/2, 5/1), and *Rosa spinosissima* (6/2), where β -carotene was in the range from 8 mg/kg to 373 mg/kg and lycopene from 13 mg/kg to 176 mg/kg, except for *R. spinosissima* (6/2) that exhibited no lycopene content. Our results are comparable with the data published by Andersson et al [27] where β -carotene ranged from 103 mg/kg to 240 mg/kg in *R. rubiginosa* and *R. dumalis* hybrid; lycopene ranged from 170 mg/kg to 220 mg/kg in *R. dumalis* and *R. dumalis* hybrid, while no lycopene occurred in *R. spinosissima*. By contrast, lycopene was detected in *R. spinosissima* fruits by Novruzov [28]. Among the total 36 analyzed genotypes, three belonged to the low-antioxidant category, *Rosa arvensis* (1/2), *R. multiflora* (1/8), and *Rosa pulverulenta* (10/10), and ranged from 32 mg/kg to 167 mg/kg for β -carotene and from 21 mg/kg to 141 mg/kg for lycopene. It was evident that there was no or least influence of carotenoid content on the total antioxidant activity of rosehips. Fruits of some of our tested genotypes contained lycopene [e.g., *Rosa alba* (15/9) and *R. prattii* (15/10)] or β -carotene (e.g., *R. spinosissima* (6/2) and *R. spinosissima* \times *Rosa reversa* (4/4)) as the only carotenoid found in rosehips. Except for *R. spinosissima* (2/10), carotenoids in Pimpinellifoliae section were clearly dominated by β -carotene. Based on Bruneau et al [1], *R. spinosissima* seems to be embedded within section Cinnamomeae rather than Pimpinellifoliae. Other separation of sections based on carotenoid patterns in rosehips was not observed. Noticeably, the carotenoid content of five different genotypes of the *R. dumalis* (3/5, D5/3, L9/1, 4/1, and L4/1) species belonging to section Caninae was in range from 21 mg/kg to 472 mg/kg. Such large variation of carotenoid content observed within the same species (keeping aside different species within the same section) shows the necessity to screen individual rose genotypes instead of relying on species identity only.

3.2.2. Tocopherols

Determination of tocopherols was carried out on HPLC–FLD using α , γ , and $\beta + \delta$ tocopherol as reference standards. Chromatographic separation of tocopherols occurring in rosehips can be found in Figure S1(b). The major representative of this group was α -tocopherol obtained in the range from 15 mg/kg to 245 mg/kg, and the total content of tocopherols was found in the range from 35 mg/kg to 255 mg/kg in *R. prattii* (15/10) and *Rosa subcollina* \times *Rosa pendulina* (7/4; Figure 2). In our study, we also analyzed rosehip genotypes *R. dumalis* (3/5 and D5/3), *R. dumalis* hybrid (9/8, L9/1, 4/1, and L4/1), *R. rubiginosa* (4/7, 14/8, D5/2, and 5/1), and *R. spinosissima* (6/2), where α -tocopherol was in range from 72 mg/kg to 226 mg/kg and the total content was from 76 mg/kg to 232 mg/kg; the results were compared with the data published by Andersson et al [9]. The tocopherol content was reported in the range from 105 mg/kg to 190 mg/kg for α -tocopherol, with the total content from 172 mg/kg to 198 mg/kg in *R. dumalis* and *R. spinosissima*. The total tocopherol content in rose genotypes *R. arvensis* (1/2), *R. multiflora* (1/8), and *R. pulverulenta* (10/10), belonging to the low-antioxidant category, ranged from 74 mg/kg to 127 mg/kg. Interestingly, a higher concentration of γ -tocopherol was observed in the samples of *R. arvensis* (1/2), *R. arvensis* (L18/1), and *R. multiflora* (1/8) genotypes [but not of *Rosa henryi* (12/6)] belonging to the section Synstylae, indicating some relation between γ -tocopherol and the section. In Barros et al's [8] published results, β - and δ -tocopherol were also analyzed in rosehip sample with 1.9 mg/kg of β -tocopherol and absence of δ -tocopherol. Based on this study, analysis of ($\beta + \delta$)-tocopherol was carried out in rosehips, with its content ranging from LOD (~ 0.16) to 30 mg/kg. Similar to γ -tocopherol, ($\beta + \delta$)-tocopherol showed a higher content in section Synstylae samples except for *R. henryi* (12/6). This leads us to the observation that the species has a significant influence on the content of tocopherols, also recorded by Andersson et al [9]. Similar to their study, we observed a high content of tocopherols in *R. spinosissima* (2/10), but the tocopherol content of two other genotypes used in our study even surpassed it (Figure 2).

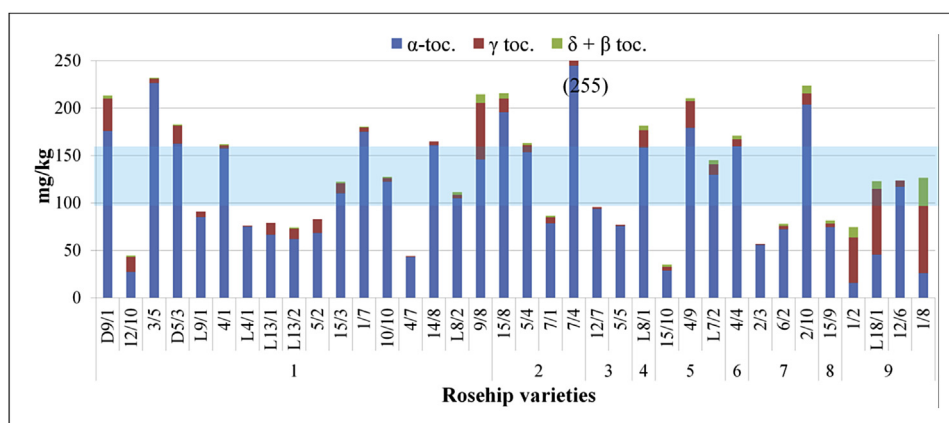


Figure 2 – Graphical representation of tocopherols in the fruits of rose genotypes. The tocopherol content is categorized based on the percentiles (mean value being 131 mg/kg): low (percentiles 0–25), moderate (percentiles 26–75) as marked in blue, and high (percentiles 76–100). Numerical labeling for rosehip sections are as follows: 1, Caninae; 2, Caninae \times Cinnamomeae; 3, Caninae \times Rosa; 4, Carolinae; 5, Cinnamomeae; 6, Cinnamomeae \times Pimpinellifoliae; 7, Pimpinellifoliae; 8, Rosa; and 9, Synstylae.

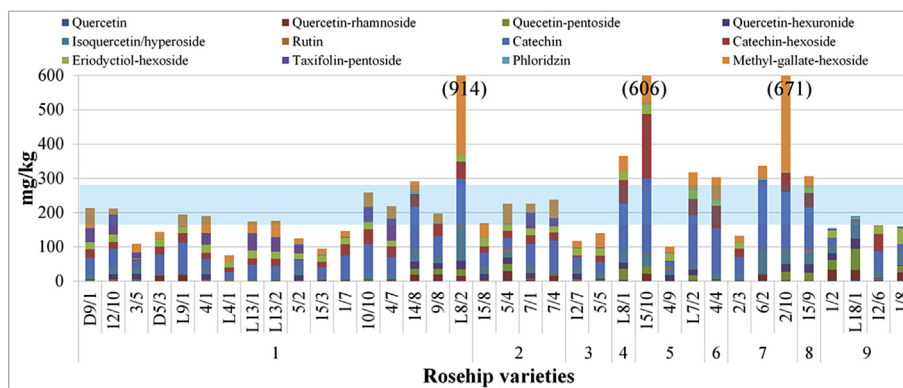


Figure 3 – Graphical representation of flavonoids/polyphenolic compounds in the fruits of rose genotypes. The flavonoid content is categorized based on the percentiles (mean value being 240 mg/kg): low (percentiles 0–25), moderate (percentiles 26–75) as marked in blue, and high (percentiles 76–100). Numerical labeling for rosehip sections are as follows: 1, Caninae; 2, Caninae × Cinnamomeae; 3, Caninae × Rosa; 4, Carolinae; 5, Cinnamomeae; 6, Cinnamomeae × Pimpinellifoliae; 7, Pimpinellifoliae; 8, Rosa; and 9, Synstylae.

3.2.3. Polyphenolic compounds/flavonoids

Using UHPLC–HRMS, the samples were screened for 12 free and conjugated polyphenolic/flavonoid compounds, the occurrence of which was reported in other studies [10,11]. In total, 15 peaks were detected in both positive and negative mode. For the total ion chromatograms along with peaks of reference standards (catechin, quercetin, and rutin), Figure S1(c) can be referred to. In some cases, for a particular *m/z* value, more than one peak was observed with different retention times. These compounds were further studied using HRMS/MS to confirm the identification of compound peaks based on fragment ions. As mentioned previously, Table 2 lists the obtained fragments along with their theoretical and acquired *m/z* values. For the *m/z* value 435.0927, corresponding to taxifolin pentoside [10,11], two compounds with similar fragmentation spectra, probably isomers, were obtained for both peaks at retention times of 3.5 minutes and 4.0 minutes. Two compounds were also detected in case of the monitored *m/z* value 449.1084, corresponding to eriodictyol hexoside [10,11]. However, in this case, fragmentation

patterns showed differences between them. All the 15 peaks were quantified or semiquantified using reference standards. The total concentration of semiquantified polyphenolic compounds/flavonoids ranged from 72 mg/kg to 914 mg/kg in *Rosa inodora* × *R. pendulina* (L7/1) and *Rosa sherardii* (L8/2; Figure 3). The LOD obtained for the reference standard rutin was ~0.06 mg/kg. Rose genotypes *R. arvensis* (1/2), *R. multiflora* (1/8), and *R. pulverulenta* (10/10) belonging to the low-antioxidant category were analyzed and semiquantified, ranging from 155 mg/kg to 259 mg/kg for the total flavonoid content. Noticeably, genotypes with a low antioxidant activity showed moderate concentration of flavonoids. Flavonoid content measured in this study is much lower compared with that reported by Sarangowa et al [29]. Rosehip samples *R. spinosissima* (2/10) and *R. sherardii* (L8/2) with 671 mg/kg and 914 mg/kg of flavonoid content, respectively, were found to have significantly higher levels of methyl gallate hexoside than other samples.

For the experimental purpose, the sample set was further reduced to 18 samples based on the antioxidant activity and

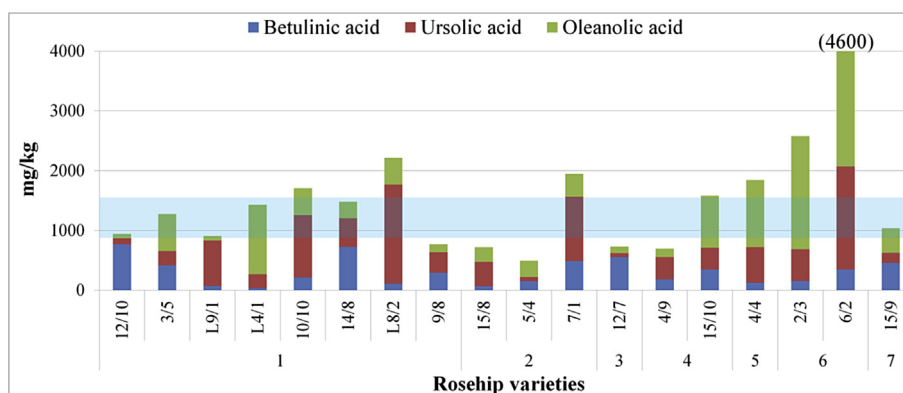


Figure 4 – Graphical representation of triterpenoic acids in the fruits of rose genotypes. The content of triterpenoic acids is categorized based on the percentiles (mean value being 1500 mg/kg): low (percentiles 0–25), moderate (percentiles 26–75) as marked in blue, and high (percentiles 76–100). Numerical labeling for rosehip sections are as follows: 1, Caninae; 2, Caninae × Cinnamomeae; 3, Caninae × Rosa; 4, Cinnamomeae; 5, Cinnamomeae × Pimpinellifoliae; 6, Pimpinellifoliae; and 7, Rosa.

previously analyzed group of compounds (carotenoids, tocopherols, and flavonoids). Out of the 18 samples (Table 1), 17 samples had a high content of the abovementioned analytes and one sample selected for comparison had a low content of the analytes. Results obtained are summarized in the following section.

3.2.4. Triterpenoic acids

Ursolic, oleanolic, and betulinic acids represent another group of targeted biologically active rosehip components that are assumed to have immunomodulatory effects [15,17–19]. For the analysis, HPLC–HRMS was used according to the method of Wenzig et al [15], as they were the first to isolate and identify triterpenoic acids in rosehips. Chromatographic separation of triterpenoic acids occurring in rosehips can be found in Figure S1(d). The content of triterpenoic acids was found in the following ranges: betulinic acid 36–772 mg/kg, oleanolic acid 66–1723 mg/kg, and ursolic acid 37–2531 mg/kg, as quantified from the negative ionization mode (Figure 4). The content of triterpenoic acid varied widely among genotypes, species, and sections with *R. spinosissima* (6/2) having the highest total triterpenoic acid content (4600 mg/kg). The sample with a low antioxidant activity and a low content of explored analytes (carotenoids, tocopherols, and flavonoids), *R. pulverulenta* (10/10), possessed a moderate content of triterpenoic acids: betulinic acid 213 mg/kg, oleanolic acid 1045 mg/kg, and ursolic acid 452 mg/kg.

3.3. Chemometric analysis

Statistical evaluation was performed to test intersectional, interspecific, and intraspecific differences among the samples. The content of flavonoids was analyzed using unsupervised PCA and supervised PLS-DA model to delineate the most important components describing data variability. Similar to Grossi et al [30], we observed that most of the *Synstylae* samples were clustered together (Figure S2) except

for *R. henryi* (12/6). This sample differed from other *Synstylae* samples in tocopherols as well. More samples of this species would be necessary to determine whether it reflects *R. henryi* genotype uniqueness within *Synstylae* or hidden hybridization of the particular studied sample. In general, large intrasectional and intraspecific variation was observed, thus blurring the clustering pattern based on sectional/species similarities. Recent molecular studies suggest that some rose sections (e.g., Cinnamomeae, Carolinae, Pimpinellifoliae, and *Synstylae*) are not monophyletic [2,31]. These findings together with an uneven number of samples in particular sections and difficulties with taxonomy identification of rose hybrids based on morphological criteria are probably responsible for the lack of more pronounced clustering in our dataset. PLS-DA plot was created with two major groups: Caninae and other (all remaining sections in one) to observe the pattern. Interestingly, the samples depicted no particular statistical pattern (Figure S3). Besides PCA and PLS-DA, Pearson's correlation coefficient was determined for assessing the antioxidant activity of the analyzed group of compounds (carotenoids, tocopherols, flavonoids, and triterpenoic acids). The *p* values were as follows: DPPH versus carotenoids, 0.035; DPPH versus Tocopherols, 0.510; DPPH versus Flavonoids, 0.053; and DPPH versus triterpenoic acids, 0.609. As can be seen from the *p* values, only carotenoids have any significant ($p < 0.05$) correlation, but participation from the rest of the components is inconclusive. This can be justified by referring to the following studies: Andersson et al [27] specified carotenoids as important antioxidants, and Hvattum [10] mentioned about flavonoids being compounds that exert antioxidant activity, while tocopherols being evident participants of antioxidant activity did not show any relation. Based on our data, we assume that different compounds contribute to the total antioxidant activity in different genotypes of rosehips, with carotenoids generally playing a greater role than tocopherols, flavonoids, and triterpenoic acids.

Table 3 – Selected rose genotypes shortlisted based on the analyzed rose hip components.^{a,b,c}

ID	Species	Section	DPPH	Carotenoids	Tocopherols	Flavonoids	Triterpenoic acids
2/3	<i>Rosa elasmacantha</i>	Pimpinellifoliae	**	*	*	*	***
L4/1	<i>Rosa dumalis</i>	Caninae	***	*	*	*	**
15/9	<i>Rosa alba</i>	Rosa	***	*	*	**	*
7/1	<i>Rosa inodora</i> × <i>Rosa pendulina</i>	Caninae × Cinnamomeae	**	**	*	**	**
4/4	<i>Rosa spinosissima</i> × <i>Rosa reversa</i>	Pimpinellifoliae × Cinnamomeae	**	*	***	**	**
15/ 10	<i>Rosa prattii</i>	Cinnamomeae	***	*	*	***	**
14/8	<i>Rosa rubiginosa</i>	Caninae	**	**	**	**	**
6/2	<i>R. spinosissima</i>	Pimpinellifoliae	**	**	*	***	***
3/5	<i>R. dumalis</i>	Caninae	**	***	***	*	**
L8/2	<i>Rosa sherardii</i>	Caninae	**	***	**	***	***

DPPH = 2,2-diphenyl-1-picrylhydrazyl.

^a The content of particular compounds/activities is categorized based on percentiles: *, low (percentiles 0–25); **, moderate (percentiles 26–75); ***, high (percentiles 76–100).

^b The mean values of particular compounds/activities were as follows: DPPH, 121–169 g/kg; carotenoids, 112–186 mg/kg; tocopherols, 98–164 mg/kg; flavonoids, 180–300 mg/kg; and triterpenoic acids, 1124–1874 mg/kg.

^c Genotypes typed in bold font are important from industrial perspective.

4. Conclusion

In this study, a large collection of rose genotypes was analyzed for the content of relevant biologically active compounds in the fruits. We detected a weak correlation between the total antioxidant activity and content of flavonoids, suggesting that flavonoids play some role in the protection of roses against oxidative stress. Carotenoids, tocopherols, and triterpenic acids were not found to correlate with the total antioxidant activity. We observed high intraspecific variation contrary to low interspecific or intersection differences in most of the analyzed compounds, which suggest limited use of the analyzed compounds for chemotaxonomic purposes. Based on the accomplished analyses, 10 best rose genotypes with the highest content of targeted biologically active compounds in the fruits were selected for further cultivation. The selected rose genotypes are shown in Table 3. As some of the selected genotypes produce small fruits (e.g., 15/10) or their palatability is low (this is especially true for all listed genotypes from section *Pimpinelifoliae*: 2/3, 6/2, and 4/4), they have only limited use in the food industry. Genotypes that are important from industrial perspective are typed in bold font (Table 3).

Declaration

Conflicts of interest

The authors declare that they have no conflict of interest.

Compliance with ethical requirements

This article does not contain any studies with human or animal subjects.

Acknowledgments

This study was carried out with financial support from the Ministry of Education, Youth and Sports, Czech Republic (project COST LD14092), the Technology Agency of the Czech Republic (project BIORAF No. TE01020080), long-term research development project of the Institute of Botany ASCR (RVO 67985939), university research is from MSMT and specific university research No. (20-SVV/2016). We would like to thank RNDr. P. Sekerka and RNDr. V. Větvicka, for their assistance in taxonomic identification, and D. Kunc, MSc, for excellent technical assistance in harvesting and processing of rosehips. Special thanks are to be given to the Botanic Garden and Gene Pool Collections of the Institute of Botany ASCR for allowing us the access to the maintained wild rose collection.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jfda.2016.12.019>.

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