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Original Article

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Assessment of Chemical and Antioxidant Properties of Fresh and Dried Rosehip (*Rosa canina* L.)

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Abstract

This work aimed to evaluate the nutritional and functional properties of rosehip from Serbia. In respect to that, the content of twenty-three elements in the rosehip along with the soil were determined by inductively coupled plasma-optical emission spectroscopy and the bioaccumulation factor (BAF) was calculated. The total dry matter, water activity, and the contents of vitamin C, total phenolics and flavonoids were determined. The antioxidant ability of fresh and dried samples was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The obtained extracts were analyzed by gas chromatography with flame ionization detection and gas chromatography – mass spectrometry techniques. In addition, assessment of the impact of thermal treatment on the chemical composition of rosehip was observed. The study revealed that the most abundant elements in rosehip were K, Ca, Mg, P and S with 2963.0, 1820.0, 709.0, 495.0 and 289.8 mg/kg, respectively. The highest BAF values in the system rosehip/soil were for S, K and P. Contents of ascorbic acid, total phenolics, total flavonoids and the antioxidant activity were reduced after the drying process by 56.3%, 20.4%, 31.3%, 21.9%, respectively. Nevertheless, dried rosehip was still a rich source of bioactive compounds with significant antioxidant activity. The presented results support traditional use of rosehip as food with health and nutritional benefits.

Keywords: antioxidant activity; bioaccumulation factor; dried rosehip; flavonoids; mineral profile; phenolics

Introduction

Rosehip is the fruit of the wild rose (*Rosa canina* L.), widespread in Europe (especially in Mediterranean area), North America, Western and Northern Asia (Nowak, 2005; Sanjust, 2008). Rosehip is a rich source of bioactive compounds with potential positive effects on human health: vitamins (B-group, C and E), carotenoids, aminoacids, organic acids (Ercisli, 2007), macro and micro elements (Damascos *et al*., 2008; Kazaz *et al*., 2009; Popović-Djordjević *et al*., 2018), as well as phenolic compounds that exhibit antioxidant, anticarcinogenic and antimutagenic properties (Tumbas *et al*., 2012). In addition, fruit contains about 30% of seeds rich in rosehip oil, which is mainly used in pharmaceutical and cosmetic industry (Concha *et al*., 2006). Studies of rosehip and rosehip oil bioactive components and their effects on the prevention of many diseases have been reported (Concha *et al*., 2006; Tumbas *et al*., 2012).

Consumption of rosehip is popular in Eastern European countries, Portugal, Germany and Scandinavian countries (Patel, 2017). Rosehips are mainly used in the food industry for the production of marmalade, jam, purée, jelly, syrup, compotes, beverages, wine, pulp, desserts, cookies, cakes etc. Besides the rich content of bioactive components, fruits have a very pleasant and exquisite aroma, which makes dried rosehip very beneficial for the production of flavour teas. Dried foods can be easily stored for a long period of time, owing to the reduction in water content. Growing interest in functional foods may provide the opportunity for the creation of a great number of products (Jones and Jew, 2007; Fan, 2014). Numerous studies about rosehip chemical and biological properties are reported (Ercisli, 2007; Demir *et al*., 2014; Taneva *et al*., 2016), however data about the chemical composition of fresh rosehip originating from different regions of Serbia are limited. In addition, evaluation of dried rosehip is barely reported (Nađpal *et al.,* 2016).

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In respect to this, the aim of the study was to characterize chemical composition of fresh and dried rosehip collected from Serbia. Mineral profile of fresh sample, total dry matter, water activity, contents of vitamine C, total phenolics, total flavonoid and DPPH radical-scavenging activity in fresh and dried samples, were determined. In addition, the assessment of the impact of the thermal treatment on the chemical compostition of rosehip was observed.

Materials and Methods

Study area

Fruits of rosehip (*Rosa canina* L.) were harvested in early October 2017 in the stage of full maturity (bright red color) in the locality of the village Rudovci (municipality of Lazarevac, Central Serbia), with coordinates 44° 22′ N/20° 25′ E, at the altitude of 209 m. Soil was taken from the same locality. The composite sample (~1 kg) was made of soil samples collected from several sites.

Trace and macro elements analysis and bioaccumulation factor

Fresh rosehip and soil were analysed for the content of macro (Ca, K, Mg, Fe, Na, Al, P, S) and microelements (As, B, Ba, Cd, Co, Cr, Cu, Hg, Li, Mn, Ni, Pb, Se, V, Zn) by means of inductively coupled plasma-optical emission spectroscopy (ICP–OES) analysis.

Hips were first washed with distilled water and then with ultra-pure water and air dried. About 0.5 g of the rosehip representative sample was destroyed with 5 ml HNO_3 (65%) and 2 ml H_2O_2 (30%) by microwave digestion (Speedwave XPERT, Microwave digestion system, BERGHOF, Germany).

The soil sample (~0.5 g) was digested in the aqua region with 15 ml of HCl (36%) and 5 ml of HNO_3 (65%) for 5 hours, at 80 °C, by microwave digestion (Speedwave XPERT, Microwave digestion system, BERGHOF, Germany). After digestion, the samples of rosehip and soil were filtered through Whatman no. 42 filter paper and diluted with ultra-pure water to the volume of 50 and 100 ml, respectively.

ICP–OES analysis was performed on the instrument Thermo Scientific, United Kingdom (model 6500 Duo, detektor CID86 chip) (Stefanović *et al*., 2016).

Bioaccumulation factor (BAF) of each element in rosehip as the ratio between its content in the fruit and the soil was calculated as reported in our previous work, according to the formula: BAF=Cf/Cs; Cf represents a concentration of the major or trace element in the rosehip fruit, while Cs stands for the concentration of the same element in the soil (Popović-Đorđević *et al*., 2018).

Antioxidant properties and phytochemical composition

Fresh and dried rosehip samples were analysed for the following parameters: total dry matter, water activity, contents of vitamine C, total phenolics (TPC), total flavonoids (TFC) and DPPH radical-scavenging activity (AA). For all the tests triplicate measurements were performed.

Dried rosehip was obtained by drying at the air temperature of 60 °C for 16 hours, and then at the air temperature of 50 °C for 20 hours. For the convective drying process, a laboratory dehydrator Stöckli with controled heater of 600 W, maintaining the set temperature of air, was used.

Total dry matter was determined by using gravimetric method (AOAC, 2005). Water activity of the samples was measured at a_w–meter TESTO 650, Germany.

Samples for vitamin C determination were prepared by using the mixure of metaphosphoric and glacial acetic acid. For all spectrophotometric methods samples were extracted with ethanol 70% (v/v). The content of vitamin C was determined by literature method based on the reversible ability of oxido-reduction system of ascorbic-dehydro-ascorbic acid (Tillmans *et al*., 1932).

Total phenolics content (TPC) was determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, 0.5 mL of diluted samples were mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 minutes. Two milliliters of sodium carbonate solution (75 g/L) was added to the mixture and then shaken. After 2 h of reaction at room temperature, the absorbance at 760 nm was measured.

The calibration curve was prepared with gallic acid solution (R^2 = 0.9982), and the results were expressed as milligrams of gallic acid equivalents on g dry weight (mg GAE/g dry weight). Triplicate measurements were performed.

Total flavonoid content (TFC) was determined using a method developed by Zhishen *et al*. (1999). Briefly, 0.5 mL of appropriately diluted sample was added to 2 mL of distilled water. At time zero, 0.15 mL of 5% $NaNO_2$ was added; at 5 min, 0.15 mL of 10% $AlCl_3$ was added; at 6 min, 1 mL of 1 mol/L NaOH was added. Afterwards, the total volume of solution was immediately made up to 5 mL with distilled water and mixed well. The absorbance was measured at 510 nm against an appropriate blank. The calibration curve was prepared with quercetin standard solutions in ethanol, and results were expressed in milligrams of quercetin equivalents on g dry weight (mg QE/g dry weight). Measurements were performed in triplicate.

DPPH (2, 2- diphenyl - 1- picrylhydrazyl) radical–scavenging activity of samples (AA) was evaluated following the procedure described by Brand–Williams *et al*. (1995). Each diluted sample (0.2 mL) was added to the DPPH working solution (2.8 mL) (mixture of 1.86×10^{-4} mol/L DPPH in ethanol and 0.1 mol/L acetate buffer (pH 4.3) in ratio 2:1). The absorbance at 525 nm was measured after the solution had been allowed to stand in the dark for 60 min. The Trolox calibration curve was plotted as a function of the percentage of inhibition of DPPH radical.

The results were expressed as millimoles of Trolox equvivalents on g dry weight (mmol TE/g dry weight). Triplicate measurements were performed.

Preparation of extracts and GC-MS analysis

Chopped and ground fruits of fresh and dried rosehip (20 g) were extracted in Soxhlet extraction system during 13 hours, using methylene-chloride (b.p. 38 °C) as a solvent. GC-MS analysis was done in duplicates. With each set of samples, a solvent blank was passed through the extraction

and GC-MS analytical procedure. The extracts were analysed by gas chromatography with flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS) techniques. The GC–FID analyses of the extracts were carried out on an Agilent 4890D gas chromatograph fitted with a HP5 capillary column (30 × 0.25 mm, 0.25 μm film), with H_2 as the carrier gas (flow rate 1 cm^3/min). All analyses were conducted in a split mode with 1:30 split ratio. Injector temperature was 250 °C, detector temperature was 300 °C, and the column temperature was linearly programmed from 40 to 280 °C (at 3 °C/min rate). The same instrumental conditions were employed for GC–MS analysis. The instrument used was an Agilent 7890N gas chromatograph fitted with a HP5–MS capillary column (30 × 0.25 mm, 0.25 μm film). The GC was coupled to a Hewlett–Packard 5972 MSD operated at 70 eV, scanning masses in the 45–550 scan range. Analyses of the extracts were conducted in a full-scan mode. The compounds were identified according to their total mass spectra, using mass spectra databases (NIST/EPA/NIH mass spectral library NIST2000, and Wiley/NBS registry of mass spectral data, 7th ed., electronic versions), and confirmed by comparison with literature data (Adams, 2007). Relative abundances of individual compounds were calculated from GC–FID peak areas.

Chemicals

Gallic acid, Folin-Ciocalteu's phenol reagent, hydrochloric acid, sodium acetate trihydrate, glacial acetic acid, aluminum chloride, sodium nitrate and sodium carbonate (anhydrous) were purchased from Merck (Darmstadt, Germany). 2, 2 -diphenyl-1-picrylhydrazyl (DPPH), 6- hydroxyl -2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) and quercetin were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium hydroxide was purchased from Fisher Scientific (Loughborough, UK). For ICP-OES analysis, ultra-pure water was prepared using the Milli-Q system (Millipore Simplicity 185 System incorporating dual UV filters (185 and 254 nm)). Nitric acid (65%), hydrochloric acid (36%) and hydrogen peroxide (30%) were used for the preparation of the samples, and were delivered from Merck, Germany. All chemicals used for the experimental procedures were of analytical grade and used as such without further purification.

Statistical analysis

Statistical analysis was performed using statistical software STATISTICA 12. The results are shown as the arithmetic mean of three replicates ± standard deviation, and the differences between individual samples were determined by t-test. Results were considered as significantly different when $p < 0.05$. Correlation analysis was carried out using the same program.

Results and Discussion

Elemental content of rosehip and soil samples

ICP-OES is an analytical method lately used for multi-elemental analysis (Cosmulescu *et al*., 2009; Stanimirović *et al*., 2018). Among 23 chemical elements analysed only Hg was not detected in both fresh rosehip and soil. Major elements of the soil were Mg and Al, followed by Ca, Mn, K, Fe, Ni and S, with other elements being presented in substantially lower content (Table 1). According to the national regulation, the content of As, Ba, Cd, Co, Cu, Pb, V and Zn were below the limit values of relevance for dangerous and harmful substances. On the other side, concentrations of Cr and Ni exceeded the limit values 1.2 and 7.5 times, respectively (Stanimirović *et al*, 2018). The vicinity of the village Rudovci to the coal mine basin Kolubara (one of the largest in Serbia) might be the reason for high concentrations of Ni and Cr in the soil. In general, the chemical composition of the plant reflects the elemental composition of the soil on which it is cultivated, and various factors impact their relations, as well as the ability of the plant to accumulate certain elements (Kabata-Pendias, 2011).

Table 1. Contents of analysed elements in rosehip and soil and bioaccumulation factor (BAF)

Element	Rosehip		Soil	BAF
	mg/kg FF	mg/100 g DW#	mg/kg	
Al	< 0.9	n.d.	15079 ± 25	/
As	< 0.005	n.d.	1.175 ± 0.004	/
B	4.86 ± 0.06	0.93 ± 0.01	13.4 ± 0.8	0.363
Ba	1.94 ± 0.009	0.370 ± 0.01	91.7 ± 0.6	0.021
Ca	1820 ± 12	348.12 ± 2	3887 ± 12	0.468
Cd	0.04 ± 0.003	0.0075 ± 0.00	0.269 ± 0.010	0.145
Co	< 0.002	n.d.	50.6 ± 0.4	/
Cr	0.006 ± 0.002	0.001 ± 0.00	121.2 ± 0.8	/
Cu	1.69 ± 0.03	0.32 ± 0.01	15.2 ± 0.6	0.111
Fe	6.39 ± 0.10	1.22 ± 0.02	472 ± 5	0.014
K	2963 ± 22	566.75 ± 4	742 ± 4	3.993
Li	< 1.2	n.d.	11.23 ± 0.08	/
Mg	709 ± 3	135.61 ± 0.6	15314 ± 25	0.046
Mn	7.66 ± 0.06	1.46 ± 0.01	1664 ± 3	0.005
Na	< 0.4	n.d.	170 ± 10	/
Ni	1.23 ± 0.008	0.236 ± 0.001	377 ± 2	0.003
P	495 ± 2	94.68 ± 0.4	1.55 ± 0.10	319.355
Pb	< 0.005	/n.d.	10.7 ± 0.6	/
S	289.8 ± 0.5	55.43 ± 0.1	309 ± 8	0.938
Se	< 0.007	n.d.	13.5 ± 0.8	/
V	< 0.080	n.d.	0.076 ± 0.003	/
Zn	2.95 ± 0.004	0.564 ± 0.00	63.0 ± 0.6	0.047

FF- fresh fruit (mg/kg); #calculated as mg per 100 g of dry weight (DW); data are expressed as mean ± standard deviation (n=3); n.d.- not detected.

In rosehip, besides Hg concentrations of Al, As, Co, Hg, Li, Na, Pb, Se and V were below the limit of detection (LOD). Concentrations of most abundant elements in rosehip were in the order K>Ca>Mg>P>S (Table 1). Due to high mobility of Cd in soil and good availability in plants, it is important to monitor its level. It was worth noting that the concentration of Cd was close to maximum allowed concentration (MAC) for this element (0.05 mg/kg) set by national regulations (Official Gazette of RS 2010/2011). Nickel is a part of nickel-containing metalloenzyme urease and may be involved in nitrogen metabolism. As reported in literature (Chizzola, 2012) the content of Ni in *Rosa canina* fruits is 0.67-2.9 mg/kg. The result obtained in our study (1.23 mg/kg) is in line with this literature report. The highest BAFs values in the system rosehip/soil were for K, P and S, whereas extremely high BAF was observed for P (319.355). More precisely, the BAF values for rosehip were in the following order: P>K>S>Ca>B>Cd>Cu>Zn> Mg>Ba>Fe>Mn>Ni.

Based on the data from the literature, the differences in the mineral composition of the rosehip from different parts of the world could be noticed (Table 2) (Damascos *et al*., 2008; Kazaz *et al*., 2009; Fan *et al*., 2014; Popović-Đorđević *et al*., 2018). The comparative data of fresh rosehip mineral content indicate that rosehip from Turkey has the richest mineral profile.

Antioxidant properties of fresh and dried rosehip

Biologically active substances with antioxidant capacity, such as ascorbic acid and phenolic compounds can be degraded during processing by thermal treatment causing a reduction of antioxidant and nutritional value of the final product (Koca *et al*., 2009). Changes during technological treatments and storage seem to be highly variable between different foods (Orphanides *et al*., 2013; Paunović *et al*., 2014). As expected, dry matter content increased in the dried sample for almost 40% (Table 3). The content of vitamin C (ascorbic acid) in dried rosehip was significantly reduced ($p < 0.05$) by 56.3% compared to the initial value in the fresh sample. This is in accordance with the results of Pirone *et al*. (2007). Ascorbic acid as a sensitive micronutrient represents a good indicator of the impact of processing condition on product quality since it can be affected by oxygen, pH, high temperatures, long processing time and cutting and maceration of the food (Oyetade *et al*., 2012).

In the present experiment, the negative effect of temperatures applied during drying processes were observed regarding the content of total phenolics and flavonoids. Namely, TPC and TFC in the dried sample decreased by 20.4% and 31.3%, respectively, compared to the fresh sample (statistically significant at $p < 0.05$) (Table 4). These results are in line with literature (Lafuente *et al*., 2011; Nađpal *et al*. 2016). A significant reduction of antioxidant activity by 21.9% ($p < 0.05$) from its initial level was also noticed, which was due to the lower contents of ascorbic acid and total phenolics in dried fruits. Even so, dried rosehip was still a rich source of bioactive compounds with high antioxidant activity, which could be contributed to the synergistic effect of vitamin C, phenolics and flavonoids (Nađpal *et al*., 2016).

Correlation matrix of studied parameters showed highly positive correlations between the content of vitamin C, TPC, TFC and AA ranging from 0.97 to 1.00 (statistically significant at $p < 0.05$).

In the preliminary assessment of phytochemical composition of fresh and dried rosehip 23 and 19, respectively, structurally diverse compounds were detected. Ketones, esters, phenols, sitosterol and alcohols were detected in both fresh and dried rosehip samples. In addition, monoterpenes and aromatic acids were present in fresh rosehip, while the absence of aldehydes, monoterpenes and aromatic acids was observed in dried fruits. Changes in phytochemical composition of rosehip fruit after drying process are presented in Fig. 1.

Table 2. The comparative data of fresh rosehip mineral content from the different regions (mg per 100 g)

Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn	mg/100 g
182.0	0.2	0.6	296.3	70.9	0.8	/	49.5	0.3	Present study
99.6	0.05	0.9	548.3	37.1	0.7	0.1	/	0.3	Serbia[a]
169	0.11	1.1	429	69	1.0	4	61	0.25	USA[b]
206.9	/	2.9	578.6	/	/	0.9	/	0.01	Argentina[c]
630.1	0.4	2.7	914.0	165.2	3.2	14.9	101.0	1.0	Turkey[d]

[#]/ – not detected or not reported; [a]- Popović-Đorđević *et al*., 2018; [b]- Fan *et al*., 2014;
[c]- Damascos *et al*., 2008; [d]- Kazaz *et al*., 2009

Table 3. Dry matter content, water activity and vitamin C content in fresh and dried rosehip

Rosehip	Dry matter (%)	Water activity	Vitamin C (mg/100g DW)
Fresh	52.28^{a}±0.76	0.91^{a}±0.01	429.55^{a}±0.64
Dried	90.33^{b}±0.51	0.42^{b}±0.01	187.67^{b}±1.25

DW–dry weight; Values are presented as means±SD (n=3); Different letters within columns indicate a significant difference at $p < 0.05$

Table 4. Total phenolics content (TPC), total flavonoid content (TFC) and antioxidant activity (AA) of fresh and dried rosehip fruits

Rosehip	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	AA (mM TE/g DW)
Fresh	90.51^{a}±0.53	38.52^{a}±0.82	0.32^{a}±0.01
Dried	72.09^{b}±1.08	26.47^{b}±1.03	0.25^{b}±0.00

Values are presented as means±SD (n=3); Different letters within columns indicate a significant difference at $p < 0.05$.

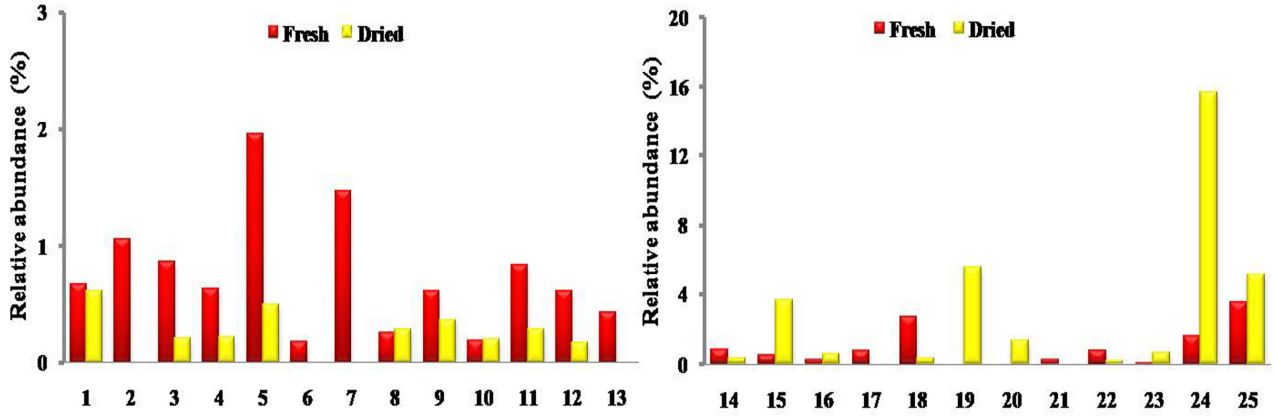

Fig. 1. Fitochemical composition of fresh and dried rosehip; **1**-6-methyl-5-hepten-2-one; **2**-2H-pyran-2,6(3H)-dione; **3**-1,2-cyclohexanedione; **4**-3-nonanone; **5**-1,3-diacetylbenzene; **6**-2-cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl- (3-oxo-α-ionol); **7**-2-cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-; **8**- maltol; **9**-2,2-dimethyl-cyclohex-3-en-1-ol; **10**-5-hydroxymethylfurfural; **11**- benzaldehyde, 4-hydroxy-; **12**- vanillin; **13**- limonene; **14**- *iso*-amyl 2-methyl butyrate; **15**-linolenic acid, methyl ester; **16**- methyl stearate; **17**- dihydro-4-hydroxy-2-(3H)-furanone; **18**-2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-; **19**- hexadecanoic acid, methyl ester; **20**-9,12-octadecadienoic acid, methyl ester; **21**- homovanillic acid; **22**-4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol; **23**- γ-tocopherol, O-methyl-; **24**- α-tocopherol; **25**- β-sitosterol

In the fresh sample, β-sitosterol (25) was the main component of fresh rosehip with the highest relative abundance (3.52%). This is one of the most commonly found phytosterol in nature and in recent years it has been the subject of numerous studies in medicine with great potential of its use for health promoting effect (Loizou *et al*., 2010). Among phenolic compounds monitored, α-tocopherol (24) was the most prevalent (Fig. 1). Its higher level (15.67%) in the dried than in the fresh sample (1.61%) indicated that this liposoluble vitamin has been relatively resistant to increased temperatures, which confirms the results of Kuppithayanant *et al*., (2014). The increased levels of certain compounds such as β-sitosterol, α-tocopherol, methyl stearate and linolenic acid methyl ester, in the dried compared to the fresh sample, may be explained by water loss during dehydration process. Compounds such as 6-methyl-5-hepten-2-one (1), 2H-pyran-2,6(3H)-dione (2) and limonene (13) detected in fresh rosehip are in line with the literature (Demir *et al*., 2014; Murathan *et al*., 2016). Generally, the majority of the compounds found in fresh rosehip were also detected in dried form. These results suggest that moderate heating applied during drying process did not entirely affect the stability of particular ketones, aldehydes, hydroxyaldehydes, esters, phenols and sterols. Interestingly, in the dried sample, methyl esters of hexadecanoic and 9,12-octadecadienoic acids (19 and 20, respectively) were detected, although they were not recorded in the fresh sample. These fatty acids esters have been identified as components of rosehip seed oil (Nowak, 2005).

Conclusions

The analysed rosehip was a rich source of K, Ca, Mg and P. Increased contents of Cr and Ni were observed in the soil. The highest BAF values in the system rosehip/soil were for K, P and S. The results showed that the dehydration process conducted at moderate temperatures significantly reduced contents of ascorbic acid, total phenolics, total flavonoids and antioxidant capacity in the fresh sample by 56.3%, 20.4%, 31.3% and 21.9%, respectively. Drying process also provoked changes in phytochemical composition and the absence of aldehydes, monoterpenes and aromatic acids was observed in dried fruits. Still, dried rosehip was a rich source of bioactive compounds with significant antioxidant activity. In order to gain a complete picture about chemical composition of fresh and dried rosehip further research is necessary. The growing interest in functional foods may provide the opportunity for the creation of a great number of products from rosehip fruit.

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