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MORPHOLOGICAL AND ANTIRADICAL CHARACTERISTICS OF RUGOSA ROSE (*ROSA RUGOSA* THUNB.) FRUITS CANNED IN DIFFERENT KIND OF HONEYS AND IN BEVERAGES PREPARED FROM HONEY

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ABSTRACT

The aim of the work was to determined the basic morphological and morphometric traits of rugosa rose (*Rosa rugosa* Thunb.) and antiradical activity of fruit pulp canned in different kind of honeys and in beverages prepared from honey. In experiments there were used 4 genotypes of roses originated from arboretum Mlyňany (Slovakia). The evaluation of 11 morphometric traits of fruit showed that the average weight of the fresh fruit without pedicle reached up 5.14 – 5.46 g, the weight of pedicle was 0.05 – 0.08 g, weight of pulp and seeds 4.80 – 5.13 g, weight of calyx 0.25 – 0.31 g, length and width of fruit (16.10 – 18.13 mm, 21.38 – 22.46 mm), the number of seeds in fruit 48.45 – 71.05, thickness of pulp 2.63 – 2.97 mm. Separated fruit pulp was canned at 40 °C and 80 °C and premixed in robinia honey and honeydew honey. Beverages were prepared by mixture of fruit pulp in honey (15 g) with cold water (150 mL). Antiradical activity was determined by DPPH method in fruit pulp (in methyl alcohol and water extracts), in honeys (black locust honey and honeydew honey) and beverages. There had been confirmed statistically significant differences in morphological traits, especially in colour and shape of fruit. Antiradical activity of fresh fruit pulp in methyl alcohol extract was determined 94.59%, in water extract 89.71%. Antiradical activity of black locust honey was 7.63%, honeydew honey 6.54%. Antiradical activity was determined also adding honeydew honey and black locust honey to fresh pulp of fruit prepared at 80 °C (33.55% and 77.58%). In beverages prepared from fresh pulp, honey and water it was investigated the higher values of antiradical activity in samples with addition of honeydew honey (81.81 – 83.86%) in comparison with robinia honey (75.57 – 79.96%).

Keywords: *Rosa rugose*; antioxidant activity; DPPH radical; honey; morphology

INTRODUCTION

In recent years, there has been increased interest in utilisation of plants in relation of prevention and health support especially in the area of chronic diseases and healthy diet. At the same time it has been noticed the progress in scientific knowledge about less-known and less used species such as *Asimina triloba* L., *Elaeagnus multiflora* Thunb., *Cornus mas* L., *Diospyros virginiana* L., *Morus nigra* L., *Pseudocydonia sinensis* Schneid., *Sambucus nigra* L., *Ziziphus jujuba* Mill. **(Brindza et al., 2007; Grygorieva et al., 2014; Grygorieva et al., 2018a; Grygorieva et al., 2018b; Monka et al., 2014; Kucharska et al., 2015; Klymenko, Grygorieva and Brindza, 2017; Ivanišová et al., 2017; Horčinová Sedláčková, Grygorieva and Brindza, 2018; Horčinová Sedláčková et al., 2018; Brindza et al., 2019)** especially in case of bioactive components displayed health benefits including *Rosa rugosa* Thunb. (rugosa rose, beach rose, Japanese rose, or Ramanas rose). Moreover, mentioned species displayed significant antioxidant potential **(Capcarova et al., 2012)**.

Rugosa rose belong to family *Rosaceae* Juss. a genus *Rosa* L. The species is native to eastern Asia, in China, Japan, Korea and south-eastern Siberia (Kamtschatka). It has been cultivated for ornamental purposes and especially valued for fragrant flowers with exotic colour in another part of world, especially in Asia and Europe **(Buchwald et al., 2007)**. In China the species has been utilised in folk medicine and food industry for about thousand years. *Rosa rugosa* is a suckering shrub 1.5 – 2 m with the bright crinkled leaves typically turn yellow before falling in autumn **(Bruun, 2005; Jung et al., 2005)**. Rugosa rose is very adaptable, with heat and drought tolerance, cold hardiness endured very low temperatures up to -45 °C. It tolerates full sun and partial shade **(Strobel, 2006)**. Moreover, it has high degree of pests and diseases resistance not accumulated heavy metals **(Calzoni et al., 2007)**. On the other hand, it is sensitive to flooding and underfooding soils. It gives regular yields, size of fruit and high degree of utilised parts of plants **(Procházka, 2007)**.

The fruit are bright, smooth red hips. The hips are large (3 – 3.5 cm), shorter in relation to fruit diameter (2 – 2.5 cm) with maximum length more than 5 cm **(Novák and Skalický, 2007)**. Hips contain 21.5% dry matter, about 1200 mg.100 g^{-1} ascorbic acid, 6.4% sugars, vitamines A, B_1, B_2, E, K and elagic acid. Fruit are valued for *β*-caroten, lycopen, tocopherol, bioflavonoids, organic acids, tannins, pectines, aminoacids and essential fatty acids content (**Novák and Skalický, 2007; Olech et al., 2012)**. The proportion of pulp takes 70 – 75% **(Najda and Buczkowska, 2013)**. In food industry fruit are valued for bioactive compounds – essential oils, flavonoids, polysaccharides, pigments and terpenoids **(Lu and Wang, 2018)**. Phenolic compounds represent the major group of biologically active substances present in rosehips, these include tannins, flavonoids, phenolic acids, and anthocyanins **(Najda and Buczkowska, 2013)**.

Rugose rose has become the widely cultivated species all around the world especially for production of flowers, fruit and other parts of plant. All parts represent the source of well known and lesser explored biologically active substances. The utilization of plant is given by the high content of bioactive substances – especially flavonoids with high antioxidant activity play an important role in prevention and treatment of cancer and diabetes. Tannins displayed antimicrobial activity. Triterpenoids (eusaphic acid, tomentic acid) revealed the pharmacological effect associated with anti-inflammatory properties. High content of ascorbic acid has been used for treatment of infectious diseases, cardiovascular diseases and metabolism disorders **(Olech et al., 2012; Lu and Wang, 2018)**. The fresh fruits are valuable for canning industry for preparation of jams, juices, sauces, syrups, wines and jellies. The regular consumption of rose hip increases the resistance of human organism against diseases. The fruit are suitable for drying and preparation of tea **(Ercisli, 2007)**. Petals are rich in essential oils, utilised in perfumes, cosmetics, aromatherapy, spices, and nutrition (tea, jams, wines and juices) **(Ma et al., 2004; Mabellini et al., 2011)**. The most important components and antiradical activity of rose hip in comparison with another rose species are given in Table 1 and Table 2. Except for rose hip very important uses has also *Rosa acicularis* Lindl. **(Sabarajkina and Brindza, 2017)**.

The rose hip has an enormous etnopharmacologic utilisation. Traditionally it has been used in treatment of *diabetes mellitus* and chronic inflammatory diseases. In Korea it has been utilised in prevention of cancer **(Cho, Yokozawa and Rhyu, 2003)**.

Methanol extract of rugose rose has been utilised in treatment of prostate cancer. **Medeiros et al. (2008)** found out that methanolic extract synergically increases the antagonistic effect of medicine from the group of anti-androgenes used in treatment of advanced stage of prostate carcinoma. Interaction of extract and medicine can give more significant effect in comparison with monocomponent utilisation **(Lee et al., 2008)**. Selective cytotoxic effects on cervical (HeLa) and breast cancer (T47D) cell lines were proved by **Olech et al. (2017a)** and **Olech et al. (2017b)**.

Antioxidant contents, antioxidant (antiradical) and anti-inflammatory properties of rosehips studied and evaluated many research works **(Gao et al., 2000; Daels-Rakotoarison et al., 2002; Böhm, Fröhlich and Bitsch, 2003; Uggla, Gao and Werlemark, 2003; Ercisli, 2007; Chrubasik et al., 2008; Barros, Carvalho and Ferreira, 2011; Guo et al., 2011; Andersson et al., 2011; Andersson et al., 2012; Zhong et al., 2016; Al-Yafeai, Bellstedt and Böhm, 2018; Al-Yafeai, Malarski and Böhm, 2018)**.

Because of unique physico-chemical and antioxidant properties the rugose rose has a good potential for processing industry. In Europe it has been mostly utilised rose hip and rugose hip in food industry **(Henker, 2000)**. Biological pecularities, broad utilisation of all part of plants, biochemical composition and verified therapeutically effect has ranged rugose hip into potential species in socio-economical development especially family farms. The plant has great importance and potential for medicine and human health and pharmaceutical and food products as well **(Olech et al., 2017a; Olech et al., 2017b)**.

This was the reason why rugose hips became the goal of research. The present study aimed at evaluating and comparing of the basic morphological, morphometric properties of rugose hip genotypes and antiradical activity of fruit pulp and especially honeys and combination of prepared products for food industry.

Scientific hypothesis

Variability of evaluated morphometric traits of rugosa rose in collection of genotypes is high.

Antiradical activity of preserved and separated pulp of matured fruit achieved by heating with addition of honey is higher *versus* pulp of matured fruit.

MATERIAL AND METHODOLOGY

Experimental material

The 4 genotypes of rugose rose originated from arboretum Mlyňany (Slovakia) in altitude 167 above sea level, with average year temperature 9.5 °C. Fruit were collected in physiological stage of maturity at the end of september. Genotypes were marked according to latin name *Rosa rugosa* (RR) from RR-01 up to RR-04.

Determination of morphometric traits

The representative sample was 30 fruit. In the collection of fruit were measured the following morhometric traits: weight of fruit (g), weight of stalk (g), weight of fruit pulp with seeds (g), weight of calyx (g), weight of seeds (g), length of stalk (mm), length of calyx (mm), length of fruit (mm), width of fruit (mm), thickness of pulp (mm) and number of seeds in fruit. Separation of individual parts was provided by mechanical separation.

Weight was measured using analytical scales (Kern ADB-A01S05, Germany) with an accuracy of 0.01 mg, length and thickness were measured using by Metrica 10002 Digital calliper with an accuracy of 0.01 mm.

Conservation of fruit pulp of rugose rose with honeys

The pulp of fruit of mature rugose rose were separated by heating at 40 °C (variant I) and 80 °C (variant II). The seeds from overcooked fruit were separated mechanically. For the preparation of samples of pulp 25% proportion from each genotype were used. The pulp was mixed with black locust (*Robinia pseudoacacia* L.) honey (M1) and honeydew honey (M2) in variable proportion of fruit pulp and honey (Table 3).

Beverages from canned fruit pulp in honey

The bevarages were prepared by mix of 15 g of samples 1 – 8 (Table 3) with 150 mL of water.

Determination of antiradical activity of fruit

The antiradical activity of fresh fruit pulp of rugose rose was determined in methanolic (RRM) and water extract (RRW). The samples 1 g in 25 mL water/methyl alcohol were mixed for 12 hours and antiradical activity was determined after filtration of samples.

In the frame of antiradical activity (ability to eliminate the free radicals) was tested the capacity of rugosa rose to remove DPPH$^•$ radicals (2.2-diphenyl-1-picrylhydrazyl) using methods of **Brand-Williams, Cuvelier and Berset (1995)** and of **Sánchez-Moreno, Larrauri and Saura-Calixto (1998)**. Absorbance at 515 nm has been registered in regular time intervals until the reaction equilibrium was reached – using the GENESYS 20 Vis Spectrophotometer (Thermo Fisher Scientific Inc., USA). First was measured the DPPH$^•$ (Sigma Aldrich, USA) absorbancy without antioxidant substance (control). The inhibition of DPPH$^•$ radicals was calculated in percent of free DPPH$^•$ radicals in the samples using the method of **Von Gadow, Joubert and Hansmann (1997)**:

% of inhibition = $[(A_{C0} - A_{At})/A_{C0}] \times 100$;

Where: A_{C0} is absorbance of control in time t = 0 min (DPPH• solution), AAt is absorbance in the presence of antioxidant in time t min, the result is in % of DPPH• radicals inhibition.

Statistic analysis

The degree of variability was determined by values of variation coefficients **(Stehlíková, 1998)**. Probability of differencies among genotypes was tested by Tukkey test in programme Statistica 13.1. Correlation analyses explored relation between fruit weight and assayed morphometric traits of fruit.

RESULTS AND DISCUSSION

Morphometric traits

Investigations of morphology of roses are still relatively few numbers **(Khapugin, 2015)**. Fruit weight represents the very important economic trait in respect of the practical utilisation of fruit. Fruit presents potential raw material for food industry. This is a reason for selection of cultivars with high proportion of pulp and lower amount of seeds and another part of fruit **(Najda and Buczkowska, 2013)**. Fruit of rugose rose are presented on Figure 1.

Weight of the fruit, pulp and seed

The results of morphometric analyses showed that the average weight of fruit ranged from 5.14 g (RR-02) up to 5.46 g (RR-01), weight of pulp with seeds from 4.8 (RR-02) to 5.13 g (RR-01). Variation coefficients confirmed the average and high degree of variability in the both evaluated traits (Table 4). It means that on shrubs are fruit of different weight. These facts were verified within tested collection by analysis of variance. There has not been proved statistically significant differencies among genotypes (Table 4). **Najda and Buczkowska (2013)** evaluated the Polish genotypes of rugose rose and found out the average weight 1.58 ± 0.16 g that represent lower value in comparison with our assayed cultivars (Table 4). The average fruit weight is very variable and influence by many factors such as cultivar, conditions of cultivation etc.

The average weight of seed of rugose rose were determined in range from 0.55 g (RR-02) up to 0.72 g (RR-03) as we can see at Table 6. Variation coefficients pointed to high degree of variability (27.47 – 50.24%). Analyse of variance confirmed statistically significant differences among genotypes with the highest value in RR-03 genotype. This genotype seems to be suitable in respect of extraction of high quality oil. **Najda and Buczkowska (2013)** found out lower values of average weigh of seed 0.19 ±0.1 g among Polish genotypes.

Shape of fruit

In the population of rugose rose has been noticed the variability in fruit shapes. Shape of fruit can be characterised by shape index, it means the ratio of length and width of fruit. The larger value of the parameter, the fruit is more elongated. The shape index of assayed genotypes varied from 0.73 – 0.84 (Table 5) that represent lower value in comparison with Polish genotypes 1.66 ±0.11% **(Najda and Buczkowska, 2013)**. The most important shapes (predominantly spherical and oval shape) are presented on Figure 1.

The evaluation of another morphometric traits

Secondly, variability of another morphometric traits (weight of fruit stalk, weight of calyx, weight of seeds, thickness of fruit pulp, average number of seeds in fruit) were determined (Table 6). Results of morphometric traits showed the high variability, so the scientific hypothesis have been proved.

Correlation analyses

The correlation coefficients were calculated among evaluated morphometric traits of rugose rose (*Rosa rugosa*) (Table 7). It has been proved positive correlation between weight of fruit and weight of pulp with seeds that is crucial for selection of fruit on maximum weight of fruit with the highest proportion of pulp with seeds (r = 0.976). It is also significant correlation between weight of fruit and weight of seeds (r = 0.613) that make possible the production of seeds with the content high quality oil. Negative correlation was evaluated between fruit weight, length of fruit and thickness of pulp. The increased size of fruit lead to the decreased thickness of pulp (r = -0.564) that was presented in Table 4 (genotype RR-03).

Table 1 The overview of total polyphenols, ascorbic acid, antiradical activity of fruit of selected species of roses **(Najda and Buczkowska, 2013)**.

Species	Total polyphenols ($mg.100g^{-1}$ FM)	Ascorbic acid ($mg.100g^{-1}$ FM)	DPPH (μM TE.g^{-1} FM)
Rosa californica	161.03 ±0.14[b]	863 ±0.1[b]	59.7 ±0.01[b]
Rosa villosa	192.56 ±0.25[a]	706 ±0.4[c]	51.3 ±0.07[c]
Rosa rugosa	**215.14 ±0.18[a]**	**974 ±0.1[a]**	**74.5 ±0.05[a]**
Rosa spinosissima	121.38 ±0.05[c]	845 ±0.2[b]	61.2 ±0.08[b]
Rosa damascene	109.67 ±0.15[c]	932 ±0.3[a]	70.4 ±0.11[a]

Note: FM – fresh mass; DPPH – antiradical activity (ability to eliminate the free radicals) was tested the capacity to remove DPPH• radicals (2.2-diphenyl-1-picrylhydrazyl). Statistical significance in case of different letters.

Table 2 The acidity, the content of extract and sugars in selected species of roses **(Najda and Buckowska, 2013)**.

Species	Acidity (%)	Extract (%)	Sugars (%)
Rosa californica	2.07 ±0.13[a]	18.2 ±0.01[b]	20.3 ±0.06[b]
Rosa villosa	0.89 ±0.02[c]	17.0 ±0.03[b]	17.1 ±0.03[b]
Rosa rugosa	**1.18 ±0.07[c]**	**20.1 ±0.01[a]**	**32.6 ±0.02[a]**
Rosa spinosissima	1.29 ±0.09[b]	19.7 ±0.04[a]	27.5 ±0.05[a]
Rosa damascene	1.21 ±0.18[b]	18.6 ±0.07[b]	21.9 ±0.01[b]

Note: Statistical significance in case of different letters.

Table 3 Assayed samples of fruit pulp of rugose rose canned with honey.

Number of sample	Abbrevation	Pulp of fruit (g) + honey (g)
	pulp from matured fruit at 40 °C	
1	RR–M_1	50 +100
2	RR–M_2	50 +100
3	RR–M_1	50 +150
4	RR–M_2	50 +150
5	RR–M_1	50 +50
6	RR–M_2	50 +50
	pulp from matured fruit at 80 °C	
7	RR–M_1	50 +100
8	RR–M_2	50 +100

Note: Genotypes marked according to latin name *Rosa rugosa* (RR) from RR-01 up to RR-04; M1 – black locust honey, M2 – honeydew honey.

Table 4 Variability of weight of fresh fruit and weight of pulp with seeds among assayed genotypes of rugose rose (*Rosa rugosa* Thunb.).

Genotypes	n	Fresh weight of fruit (g)					Weight of pulp and seeds (g)					Proportion of weight of pulp and seeds (%)
		min	max	x	s_x	V%	min	max	x	s_x	V%	
RR-01	30	3.40	8.41	5.46[a]	0.35	28.73	3.10	8.01	5.13[b]	0.34	29.63	93.58
RR-02	30	2.08	7.33	5.14[a]	0.27	24.08	1.83	6.74	4.80[a]	0.26	24.50	93.38
RR-03	30	3.85	7.62	5.43[a]	0.22	18.74	3.38	7.12	5.03[a]	0.22	19.86	92.63
RR-04	30	2.91	9.09	5.18[a]	0.30	26.63	2.68	8.71	4.88[a]	0.30	27.53	94.20

Note: n – total number of evaluated fruit; min – minimal value; max – maximal value; x – mean value of set of genotypes; s_x – standard error of the mean; V % – variation coefficient in %; proportion of weight of pulp and seeds from total weight of fruit (%) – percentual proportion of weight of separated parts of fruit in relation to average weight of fruit.

Table 5 Variability of lenght and diameter of fruit (mm) among genotypes of rugose rose (*Rosa rugosa* Thunb.).

Genotypes	n	Length of fruit (mm)					Width of fruit (mm)					Length/width ratio (L/W)
		min	max	x	s_x	V%	min	max	x	s_x	V%	
RR-01	30	12.23	22.14	16.42[a]	0.60	16.52	18.57	27.50	22.46[a]	0.54	10.76	0.73
RR-02	30	14.28	26.26	18.13[a]	0.68	16.78	15.58	24.27	21.58[a]	0.42	8.70	0.84
RR-03	30	12.89	20.01	16.10[b]	0.45	12.48	17.75	25.80	21.38[a]	0.49	10.31	0.75
RR-04	30	12.05	20.42	16.41[a]	0.48	13.22	17.78	27.04	21.42[a]	0.51	10.68	0.76

Note: n – total number of evaluated fruit; min – minimal value; max – maximal value; x – mean value of set of genotypes; s_x – standard error of the mean; V % – variation coefficient in %.

Table 6 Variability of another selected morphometric traits of rugose rose (*Rosa rugosa* Thunb.) in collection of genotypes.

Genotypes	Weight of fruit stalk (g)		Weight of calyx (g)		Weight of seeds (g)		Thickness of fruit pulp (mm)		Average number of seeds in fruit (pcs)	
	x	V%	x	Cv%	x	V%	x	V%	x	V%
RR-01	0.05[b]	29.32	0.27[b]	19.89	0.70[a]	46.16	2.67[a]	24.37	11.78[c]	40.79
RR-02	0.05[b]	77.21	0.28[a]	16.42	0.55[b]	50.24	2.97[a]	18.15	15.10[a]	40.87
RR-03	0.08[a]	47.47	0.31[a]	18.07	0.72[a]	27.47	2.67[a]	16.35	14.80[a]	23.85
RR-04	0.05[b]	38.76	0.25[b]	24.29	0.71[a]	37.90	2.63[a]	15.39	13.38[b]	35.37

Note: x – mean value; V% – variation coefficient in %; pcs – pieces; Statistical significance in case of different letters. Genotypes marked according to latin name *Rosa rugosa* (RR) from RR-01 up to RR-04.

Table 7 Correlation analyses among morphometric traits of rugose rose (*Rosa rugosa* Thunb.).

r	s_r	CO	r^2	t-test	Probability
		weight of fruit (g) : weight of stalk (g)			
0.491	0.014	$-0.890 \leq r \geq 0.986$	0.241	0.797	0.509
		weight of fruit (g) : weight of fruit pulp and seeds (g)			
0.976	0.041	$0.229 \leq r \geq 0.999$	0.952	6.264	0.024
		weight of fruit (g) : weight of calyx (g)			
0.459	0.031	$-0.898 \leq r \geq 0.985$	0.211	0.732	0.541
		weight of fruit (g) : weight of seeds (g)			
0.613	0.079	$-0.846 \leq r \geq 0.990$	0.376	1.099	0.386
		weight of fruit (g) : length of stalk (mm)			
-0.475	1.640	$-0.986 \leq r \geq 0.894$	0.226	0.764	0.524
		weight of fruit (g) : lenght of calyx (mm)			
0.772	1.742	$-0.734 \leq r \geq 0.994$	0.595	1.714	0.228
		weight of fruit (g) : length of fruit (mm)			
-0.689	0.817	$-0.993 \leq r \geq 0.8054$	0.4748	1.344	0.311
		weight of fruit (g) : width of fruit (mm)			
0.541	0.524	$-0.875 \leq r \geq 0.988$	0.292	0.908	0.459
		weight of fruit (g) : number of seeds (pieces)			
0.949	3.826	$-0.130 \leq r \geq 0.999$	0.902	4.291	0.050
		weight of fruit (g) : thickness of fruit pulp (mm)			
-0.564	0.161	$-0.989 \leq r \geq 0.867$	0.3180	0.966	0.436

Note: r – correlation coefficient, s_r – standard error of the correlation coefficient, CO – correlation, r^2– coefficient of determination.

Antiradical activity of fruit pulp

Extracts of fruit pulp were prepared in two versions: water and methanolic. Previous studies **(Dudra et al., 2015; Olech et al., 2017a; Olech et al., 2017b)** confirmed that extracts of *Rosa rugosa* displayed strong antioxidant and antiradical activity (up to EC50 0.85 mg.mg^{-1} DPPH) indicating widespread utilisation of rugose rose as natural antioxidant in food and pharmaceutical industry. In same way our results pointed to strong antiradical activity of aqueous 90.22 – 90.84% and methanolic extract as well 92.87 – 94.59% (Table 8). The notable antioxidant activity was also confirmed *Rosa dumalis* Bechst., *R. dumetorum* Thuill. and *R. sempervirens* LM **(Nadpal et al., 2018)**.

There has not been proved statistically significance between assayed extracts. **Dudra et al. (2015)** compared antioxidant activity of *Rosa canina* fruit in relation to type of solvent and found out that ethylacetate and water extract showed lower values of antioxidant activity than methanolic and diethyleter extracts. Similarly, **Olech and**

Nowak (2012) noticed the significant influence of extraction technique on antiradical activity of rugose rose petals. According to results of experiment they reccomended polar organic solvents. High efficacy of 15% methanolic extract of rugose fruit was proved in respect of antiradical activity in studies of **Lee et al. (2008)**.

In study of **Al-Yafeai, Bellstedt and Böhm (2018)** different degrees of ripeness affected the bioactive compounds as well as the antioxidant capacity in the fruit of *R. rugosa* (hips). The maximum concentration of carotenoids was observed at late harvesting. The maximum concentration of both vitamin E and vitamin C was obtained in the orange hips and total phenolic contents were determined in the mature hips (red colour) with significant difference. The highest hydrophilic and lipophilic Trolox equivalent antioxidant capacity (TEAC) values were determined in the mature red hips, whereas oxygen radical absorbance capacity (ORAC) showed significantly lower activity in the mature hips. **Andersson et al. (2012)** found that amounts of total tocopherols and vitamin E activity being decreased in rose hips during ripening. According to **Uggla, Gao and Werlemark (2003)** the contents of vitamin C in rosehips ranged from 200 to 2800 mg.100g^{-1}, rosehips are considered the most abundant source of natural vitamin C. **Ercisli (2007)** found that contents of ascorbic acid (vitamin C) in the fresh fruits of rose species were between 706 and 974 mg.100g^{-1}. According to **Al-Yafeai, Bellstedt and Böhm (2018)** the contents of ascorbic acid in *R. rugosa* hips at different ripening degrees ranged between 798 and 1090 mg.100g^{-1}. The medicinal value of rosehips depends largely on the vitamin C contents.

Table 8 Antiradical activity (%) of pulp of rugose rose (*Rosa rugosa* Thunb.).

Sample	min	max	x	s_x	V%
RRW	90.22	90.84	90.47^a	0.19	3.65
RRM	92.87	94.59	93.73^a	0.49	9.15

Note: min – minimal value; max – maximal value; x – mean value of set of genotypes; s_x – standard error of the mean; V % – variation coefficient in %; RRW – fresh fruit pulp of rugose rose in water extract; RRM – fresh fruit pulp of rugose rose in methanolic extract.

Table 9 Antiradical activity (%) of assayed honeys.

Honey	n	min	max	x	s_x	V%
Black locust	5	6.35	8.35	7.63^a	0.645	14.62
Honeydew	5	6.11	7.28	6.54^a	0.373	9.85

Note: n – number of samples; min – minimal value; max – maximal value; x – mean value of set of genotypes; s_x – standard error of the mean; V % – variation coefficient in %.

Antiradical activity of honeys

Black locust honey and honeydew honey were used for conservation of fruit pulp of rugose rose. Antiradical activity of black locust honey and honeydew honey represented 6.35 – 8.35% and 6.11 – 7.28% respectively. There have not been statistically significant differences between assayed honey types (Table 9).

Antiradical activity of fruit pulp preservated in honey

The average values of antiradical activity of fruit pulp preserved in honey ranged from 33.55% (sample 8) up to 77.58% (sample 1) as it has been showed at Table 10. Addition of honey caused decrease antiradical activity of fruit pulp. On the other hand, honey displays unique antimicrobial properties **(Israili, 2014; Faustino and Pinheiro, 2015)**. The addition of honey to samples leads to stabilization of final products and play an important role as natural sweetener. The high temperature 80 °C during separation and mix of fruit pulp cause the degradation of thermolabile compounds that generally lead to decrease of antiradical activity after addition of preservants by 36% (black locust honey) and by 41% (honeydew honey). The analyse of variance confirmed the statistically significant

Table 10 Antiradical activity (%) of pulp and beverages with fruit pulp of *Rosa rugosa* Thunb. preserved in black locust honey and honeydew honey.

Number of sample	Sample	Honey	Fruit pulp (g) + honey (g)	x	s_x	V%	x	s_x	V%
	Preserved and separated pulp of matured fruit achieved by heating at 40 °C with addition of honey						**Beverages**		
1	RR-M$_1$	black locust	50 + 100	77.58^a	2.26	5.06	79.96^b	4.08	8.85
2	RR-M$_2$	honeydew	50 + 100	57.79^b	3.28	9.84	83.63^a	2.50	5.18
3	RR-M$_1$	black locust	50 + 150	56.90^b	3.26	9.94	79.25^b	1.86	4.06
4	RR-M$_2$	honeydew	50 + 150	66.86^a	2.95	7.65	81.92^a	3.88	8.20
5	RR-M$_1$	black locust	50 + 50	65.38^a	0.88	2.34	76.90^b	5.47	12.32
6	RR-M$_2$	honeydew	50 + 50	70.96^a	4.18	10.20	83.29^a	3.53	7.34
	Preserved and separated pulp of matured fruit achieved by heating at 80 °C with addition of honey						**Beverages**		
7	RR-M$_1$	black locust	50 + 100	48.98^c	3.76	13.30	75.57^b	0.89	2.05
8	RR-M$_2$	honeydew	50 + 100	33.55^d	0.74	3.86	81.81^a	0.97	2.05

Note: n – total number of evaluated fruit; min – minimal value; max – maximal value; x – mean value of set of genotypes; s_x – standard error of the mean; V % – variation coefficient in %; M1 – black locust honey, M2 – honeydew honey. Statistical significance in case of different letters.

Figure 1 Fruit of rugosa rose (*Rosa rugosa* Thunb.).

differences among assayed samples, variation coefficient ranged from 2.34 – 13.30% pointed to low up to high degree of variability.

The antimicrobial activity of bee honey is one of its most studied biological properties. The specificity of this activity, as well as the others honey's bioactivities such as antitumor, anti-inflammatory, antioxidant and antiviral properties, depends on honey's components, which vary according to its floral, geographical and entomological origin **(Kačániová and Almeida-Aguiar, 2016)**. **Kačániová et al. (2009)** study the antimicrobial activity of honey samples against *Candida species.* The antimicrobial activity was determined as an equivalent of the inhibition zones diameters (in millimetres) after incubation of the cultures for 48 hours. There were not seen an inhibition zones against the yeasts investigated in the 25 and 50% concentration of honey samples. In another study **Kačániová et al. (2011)** the antifungal activities of honey samples were tested by 10, 25 and 50% (by mass per volume) concentration against fungi *Penicillium crustosum, P. expansum, P. griseofulvum, P. raistrickii* and *P. verrucosum* and by the agar well diffusion method. The solutions containing 10% (by mass per volume) of honey did not have any effect on the growth of fungi. The strongest antifungal effect was shown by 50% honey concentration against *P. raistrickii*.

Antiradical activity of beverages with pulp preserved in honey

Beverages were prepared by mixing of 15 g separated fruit pulp preserved in honey with 150 mL of water. The antiradical activity of beverages reached up 75.57% (sample 7) up to 83.63% (sample 2). The samples 7 and 8 displayed statistically significantly lower variability in antiradical activity in comparison with the rest of samples. Variation coefficients (Table 10) pointed to low or medium degree of variability (2.05 – 12.32%). Samples with addition of honeydew honey displayed the antiradical activity about 80%. Antiradical activity of beverages with pulp preserved in honey displayed higher values of antiradical activity in comparison with maturated fruit. The second hypothesis has been verified.

Total water-soluble antioxidants were also significantly varied among Rosa species. This value was significantly higher (up to 2 times) for the fruit of *Rosa rugosa* (p <0.05) **(Czyzowska et al., 2015)**.

Horčinová Sedláčková, **Grygorieva and Brindza (2018)** determined antioxidant activity of activated beverages of dilute/aqueous honey prepared from fresh inflorescences elderberry (*Sambucus nigra* L.) in the saccharide extract. It was determined antioxidant activity in the range from 16.81 to 24.16%. The enlarging of activation positively correlated with an increasing in antioxidant activity.

CONCLUSION

Rugose rose belong to prospective species for cultivation in monoculture especially in case of small and family farmers contributed to social-economic development. This species give a very valuable fruit – hips that can be utilized in preparation of teas, jams, alcoholic and non-alcoholic beverages, oils and another products. Morphological evaluation of results proved statistically significant differences in colour and shape of fruit. In the fruit pulp there has been determined a higher values of antiradical activity in methanolic extract (94.59%) than in water extract (89.71%). The average value of antiradical activity of black locust honey reached up 7.63%, and honeydew honey 6.54%. The average values of antiradical activity of fruit pulp ranged from 33.55% (canned in honeydew honey at 80 °C) up to 77.58% (in black locust honey at 40 °C). In beverages from pulp, honey and water there have been evaluated the higher values of antiradical activity in samples with addition of honeydew honey (81.81 – 83.86%) in comparison with addition of black locust honey (75.57 – 79.96%).

Fruit pulp contains the large number of bioactive components with positive nutritional and phytotherapeutic effect that has been confirmed by results of antiradical activity of fruit. In technologies of jams, syrups and other products preparations there have been utilised a high temperatures leading to degradation of thermolabile bioactive compounds. This was the reason why our research work was aimed at evaluation of different methods of hip's pulp separation to minimalize the losses. Results of determination of antiradical activity confirmed the high retention of valuable substances. Separated fruit pulp represents the initial material for production of valuable food commodities. At the same time it has been confirmed the value of traditional beverage based on water with honey that has been utilised a long time ago by ancestors. Water mixed with honey has been represented the unique natural beverage with the significant source of energy and valuable nutritional and therapeutic compounds of honey. The nutritional and therapeutic effect of honey and beverages can be increased by conservation of rugosa rose pulp without thermic treatment. Blocking of fermentation process after the addition of pulp into the honey can be solved by appropriate portion of honey and pulp.

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