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EVALUATION OF THE ROSE HIPS OF *ROSA CANINA* L. AND *ROSA RUGOSA* THUNB. AS A VALUABLE SOURCE OF BIOLOGICAL ACTIVE COMPOUNDS AND ANTIOXIDANTS ON THE BALTIC SEA COAST

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Abstract

The work involved the study of the contents of ascorbic acid, carotenoids, total flavonoids, total tannins, total phenolics, and antioxidant properties of rose hips of two species: *Rosa canina* L. and *Rosa rugosa* Thunb. collected from wild bushes growing on the Curonian Spit, Sambia peninsula, and the Vistula Spit (Kaliningrad region, Russia). Species-specific accumulation of ascorbic acid, carotenoids, flavonoids, phenolics, and total antioxidants was shown. The content of the above components was significantly higher in hips of *Rosa rugosa* than in rose hips of *Rosa canina*. With the exception of the total content total water-soluble antioxidants and flavonoids, the content of biologically active compounds was higher in fully ripe fruits compared to unripe and half-ripe fruits. The accumulation of studied phytonutrients depended also on growth locations. In this study was shown high nutritional value of rose hips, especially of the species *Rosa rugosa* Thunb.

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Introduction

Finding new natural sources of antioxidants remains an important issue, as this will not only enable to enhance the food quality but also to generally improve people's life, wellbeing, and health. The impaired natural balance of the free radical oxidation rate and the antioxidant protection activity of human body that may be caused by the adverse conditions (environmental pollution, chronic emotional stress, high content of fast digesting carbohydrates and fats in the diet with simultaneously lowered amount of bio-antioxidants) is the important factor in pathogenesis of many diseases, including cardiovascular disorders, cancer, neurodegenerative and endocrine diseases (HALLIWELL 2012, FIEDOR and BURDA 2014, CIRILLO et al. 2016).

In the endeavours to find antioxidant producing plants, of particular interest are the fruits of rose plants (*Rosa* sp.), which have been long used as raw material for medications, vitamins, and food. Rose hips have been discovered to be rich in polyphenols (mainly flavonoids including proanthocyanidins and catechins), triterpene acids, essential fatty acids, galactolipid, folate, vitamin A, C and E, mineral (Ca, Mg, K, S, Si, Se, Mn and Fe), among other bioactive components (PATEL 2017). They can be used not only for direct consumption but also for the extraction of active ingredients that can be used as food additives (JIMÉNEZ et al. 2017). Rose hips are usually harvested in late summer, early autumn or in autumn. The content of biologically active compounds is proved to depend on a number of factors, i.e. species specificity (KAZAK et al. 2009, CZYŻOWSKA et al. 2015), ripening stage (NOJAVAN et al. 2008, ADAMCZAK et al. 2012, ELMASTAŞ et al. 2017), environmental growing conditions (CHUPAKHINA et al. 2014), etc.

In phytomedicine, the most common species is *Rosa canina* L. Medicinal properties are found in flowers, hips, leaves and roots of the plant. Wild rose is used for making decoctions, juice, and jam. The plant contains vitamin C known for its immunostimulating activity, as well as A, K, E, P, and many valuable substances (BARROS et al. 2011, ŽIVKOVIĆ et al. 2015). It is also known for its ability to lower blood pressure and improve human body's resistance to colds and infections (İLBAY et al. 2013, FAN et al. 2014).

However, there has been recently a recurring discussion in various sources about curative and nutritive properties of *Rosa rugosa* Thunb. (ALTINER and KILIÇGÜN 2008, OLECH et al. 2012, DUDRA et al. 2016). In the areas on the Baltic Sea coast it is an invasive species (BRUUN 2005). Massive introduction of *Rosa rugosa* on the Baltic coast was mainly performed as part of the shore protection. Planting of these shrubs in the Curo-

nian Spit and Vistula Spit in the Kaliningrad region, for example, helped in the stabilization of sand dunes. Yet, in many European countries there have been recently a number of debates over the insistent need to control *Rosa rugosa*, being considered an active invasive species. Researchers in the Baltic countries are concerned that the plant is rapidly spreading and, establishing a dense canopy, changes natural (habitual) landscape (due to the change in phytocoenosis) (ISERMANN 2008, PROVOOST et al. 2011, DOODY 2012). So in Northwest Europe, *Rosa rugosa* forms dominant, large, and dense scrub that excludes, at a local scale, native species (ZHANG et al. 2018). And yet, before any final decision can be taken, numerous valuable qualities of *Rosa rugosa* should be thoroughly weighed, such as its curative value, vitamin content and nutritional value.

With this in view, the aim of this research was to study antioxidant properties of rose hips of two species (*Rosa canina* L. and *Rosa rugosa* Thunb.) collected from different locations at the Baltic Sea coast (in Kaliningrad region) in depending on ripening stage.

Materials and Methods

Chemicals

Trolox, quercetin, gallic acid, 2,6-dichloroindophenol, metaphosphoric acid were purchased from Acros Organics (New Jersey, USA). L-ascorbic acid, Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tripyridyls-triazine (TPTZ) were purchased from Sigma (St. Louis, MO, USA). All other reagents and solvents were analytical grade from Vecton (Russia).

Plant material

The rose hips (pseudofruits) of *Rosa canina* L. and *Rosa rugosa* Thunb. were collected from beginning of August to the end of September 2016 from wild bushes growing in three different locations on the Baltic Sea coast in Kaliningrad region (Russia). The places of collection are represented on the map (Figure 1). The rose hips were collected at three ripening stages: 1) unripe (green and hard), according BBCH scale stage 79 709 Fruits have reached 90% of final size, 2) half-ripe (orange and hard) according BBCH scale stage 85 805 Progression of cultivar-/species-specific fruit colouring: increasing intensity of colour, and 3) fully ripe (red and soft), according BBCH scale stage 88 808 Full ripeness: cultivar-/species-specific

fruit colouring and seed ripeness (MEIER et. 2009). The fruits were collected from six bushes in each location. From each bush were taken five rose hips that were combined to one sample.

Fig. 1. The map showing three places in Kaliningrad region (Russia) where rose hips (pseudofruits) of *Rosa canina* L. and *Rosa rugosa* Thunb were collected. Point *A*: Location 1 – Curonian Spit; Point *B*: Location 2 – Sambia peninsula (the fruits were collected on the north part of peninsula, close to village Roshchino); Point *C*: Location 3 – Vistula Spit

Rose hips without calyxes were washed several times with water, and the seeds of the pseudofruits were cleaned. Approximately 20–50 g of fruits samples from each bush was lyophilized (FreeZone Triad, Labconco, USA) and stored at –20°C until analysis (Liebherr, Germany). The vitamin C content was measured in fresh fruits directly on the day of collection. All other analyzes was performed in lyophilized samples.

Ascorbic acid

Ascorbic acid was determined as described by BARROS et al. (2010). Plant extract preparation: 0.15–0.20 g fresh plant material was homogenized with 10 mL of 1% metaphosphoric acid and centrifuged at 4500 rpm for 15 min at 4°C. The supernatant (1 mL) was mixed with 2,6-dichloroindophenol (9 mL) and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of L-ascorbic acid, and the results were expressed as mg of ascorbic acid per g of dry weight.

Carotenoids

Accurately weighted 0.5 g lyophilized plant sample was taken, and homogenized in tissue homogenizer (Ultra-Turrax Tube Drive, IKA, Germany) with 10 mL of 80% acetone. Homoginized sample mixture was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant were separated and 0.5 mL of it is mixed with 4.5 mL of the solvent. The optical density of the above mixture was determined at 470 nm, 646.8 nm and 663.2 nm (UV-3600, Shimadzu, Japan). The concentration of carotenoids was calculated according to (SUMANTA et al. 2014). Finally the content of carotenoids in plant was converted to mg per gram dry weight.

Total flavonoids content (TFC)

The total flavonoids content was measured by the $AlCl_3$ colorimetric assay according described by SEVKET et al. (2016). An aliquote (0.1 mL) of methanolic plant extracts was added to 10 mL volumetric tube containing 4 mL dH_2O. Then, 0.3 mL 5% $NaNO_2$ solution was added. After 5 minutes 0.3 mL 10% $AlCl_3$ solution was poured into the flask and maintained for another 6 minutes, after which 2 mL 1 M NaOH solution was added. The total volume was completed up to 10 mL with dH_2O. The solution was mixed and the absorption was measured at 510 nm (UV-3600, Shimadzu, Japan). The total flavonoids content was calculated using a calibration curve, and then expressed as mg quercetin equivalent (QE) per gram dry weight.

Total tannins content (TTC)

The total tannins content was determined by the Prussian Blue method with some modifications (GUPTA and VERMA 2011). Plant extract preparation: 0.2–0.5 g lyophilized plant material was homogenized with 10 mL of extraction solvent (1% HCl ethanol solution). The extracts were centrifuged for 30 min at 4500 rpm. Supernatants were collected and stored at 5–8°C in dark until analysed. 250 µl of extracts was taken. It was diluted within 25 mL of distilled water and added 3 mL of 0.5 M $FeCl_3$ in 0.1 N HCl and 3 mL of 0.008 M $K_3Fe(CN)_6$. Colour develops immediately after 10–15 min. The optical density of the above solution was determined at 720 nm (UV-3600, Shimadzu, Japan). The calibration curve was made by preparing gallic acid solutions at different concentrations in ethanol. The total tannins contents are expressed in mg of as gallic acid equivalent (GAE) per gram dry weight.

Total phenolic compounds content (TPC)

Phenolic compounds were extracted from lyophilized fruits with 96% ethanol solution and determined by Folin-Ciocalteu method (PADHI et al. 2017). Briefly, 100 µL of gallic acid standard or plant extract was mixed with 300 µL 0.2 M Folin-Ciocalteu reagent in a tube, and incubated for 10 min at room temperature in darkness. Next, 6 mL of 6.75% sodium carbonate (Na_2CO_3) solution was added to each tube, and the tubes were incubated for 30 min at room temperature in darkness. The optical density of the above solution was determined at 765 nm (UV-3600, Shimadzu, Japan). TPC was expressed as mg gallic acid equivalent per gram dry weight.

Total water-soluble antioxidants content (TWAC)

The total water-soluble antioxidants content have been estimated by an amperometric method using a TsvetYauza-01-AA (NPO Khimavtomatika Inc., Moscow, Russia) according to YASHIN (YASHIN 2008). Plant extract preparation: 0.2–0.5 g of lyophilized plant material was homogenized with 50 mL of eluent (solution of phosphoric acid with the molar concentration of 2.2 mM). The mixture was then filtered and used for analyse in day of preparation. The calibration curve was made by preparing quercetin solutions at different concentrations. The total antioxidant contents are expressed in mg of as quercetin equivalent (QE) per gram dry weight.

Total antioxidant capacity (TAC)

The total antioxidant capacity was measured using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, $ABTS^{\cdot+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6- sulfonic acid) radical and FRAP (ferric reducing antioxidant power) assays. Plant extract preparation: 0.1–0.2 g lyophilized plant material was homogenized with 10 mL of 96% ethanol solution.

Each extract of rose hip fruits was mixed with 2.85 mL freshly prepared 0.1 mM solution of DPPH in ethanol. The sample was incubated for 30 min at room temperature in darkness. The reduction of absorbance at 515 nm (UV-3600, Shimadzu, Japan) was measured spectrophotometrically (TÕNUTARE 2015).

ABTS and FRAP assays was performed as described by TANEVA et al. (2016). ABTS radical was generated by mixing aliquot parts of water solution of 7.0 mM (ABTS) and 2.45 mM potassium persulfate. For the assay, 2.85 mL of this $ABTS^{\cdot+}$ solution was mixed with 0.15 mL of obtained

extracts. After 15 min at 37°C in darkness the absorbance was measured at 734 nm (UV-3600, Shimadzu, Japan) against ethanol.

The FRAP reagent was freshly prepared by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6- tripyridyls-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3 \cdot 6H_2O$ in dH_2O. The reaction was started by mixing 3.0 mL FRAP reagent with 0.1 mL of investigated extract. The reaction time was 10 min at 37°C in darkness and the absorbance was measured at 593 nm (UV-3600, Shimadzu, Japan) against blank prepared with ethanol.

All results from the determination of antioxidant capacity were expressed as µmol Trolox equivalents (µmol TE) per gram dry weight.

Statistical analysis

All analyses were performed in five repetitions. The results of analyses were reported as mean ± standard deviation (SD). Three-way analysis (ANOVA) was performed using the SigmaPlot 12.3 (Systat Software GmbH, Erkrath, Germany). Before ANOVA data were checked for normality and the homogeneity of variance. Statistical comparisons were performed using the Tukey's multiple comparison test. Differences were considered significant at $p \leq 0.05$. A correlation analysis based on Pearson's chi-squared test was conducted. Similarity in the content of biological active compounds and antioxidants due to species, ripening stages and growth locations was evaluated by hierarchical cluster analysis by Ward's method in Stata v.13, using the Euclidean distance as a similarity measure. The reliability of clusters was evaluated by bootstrapping with 1000 replicates in PAST v. 3.17.

Results

Ascorbic acid

This study proved species-specific ascorbic acid accumulation. Vitamin C content was evidently higher in *Rosa rugosa* fruits ($p \leq 0.05$), which contained Vitamin C at over 40 mg g^{-1} dry weight (Figure 2 , Table appx. 1). The content of ascorbic acid in rose hips depended also on the stage of their ripening. The maximum level was determined in the fruits collected at the stage of full ripeness. At the same time, the unripe and half-ripe fruits of *Rosa canina* were characterized by almost the same level of vitamin C, while its content in the unripe fruits of *Rosa rugosa* was significantly lower compared to fully ripe and half-ripe fruits (Figure 2). The results of the

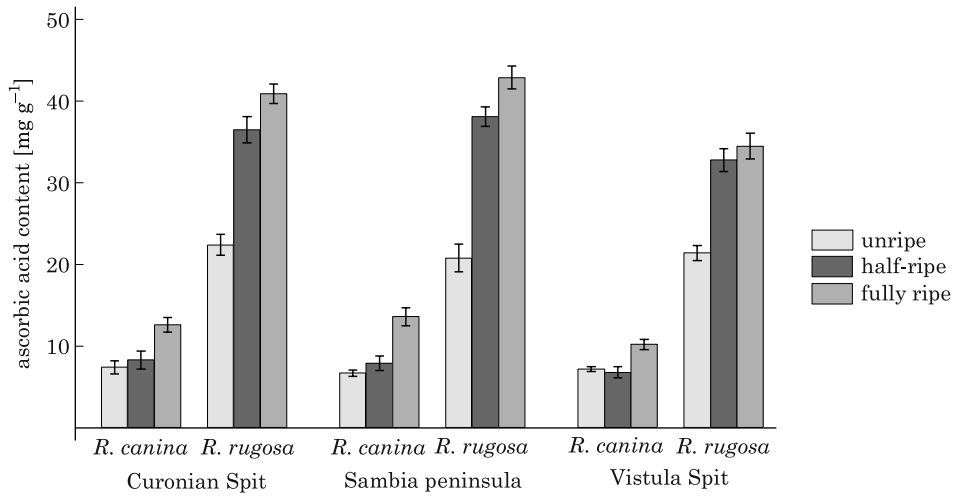

Fig. 2. Ascorbic acid content in fruits of *Rosa canina* and *Rosa rugosa* collected at different ripening stages and from three growth locations

evaluation via 3-factorial ANOVA (Table appx. 1) showed that growth location has a significant effect on the ascorbic acid content in rose hips. A lower content of ascorbic acid was found in the fruits collected on the Vistula Spit. Significant differences between the fruits collected in two other places were not identified.

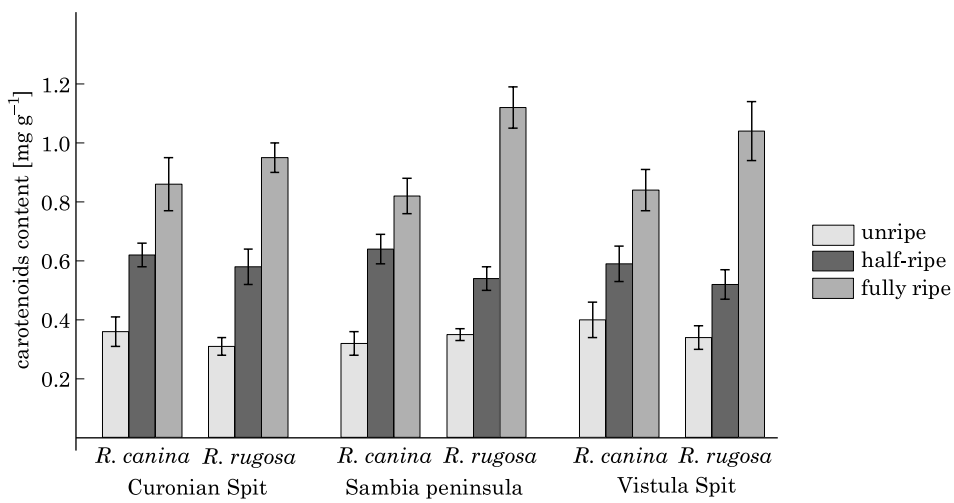

Fig. 3. Carotenoids content in fruits of *Rosa canina* and *Rosa rugosa* collected at different ripening stages and from three growth locations

Carotenoids

As shown in Figure 3 , the fruits of *Rosa canina* and *Rosa rugosa* had nearly the same content of carotenoids (0.61–0.64 mg g^{-1}). However, a statistically significantly higher content of carotenoids was established in the fruits of *Rosa rugosa* (Table appx. 1). The stage of ripeness was the decisive factor that determined the level of carotenoids in rose hips of both species. The level of carotenoids in fruits increased up to three times during their ripening. The growth location had no significant effect on the content of carotenoids.

Phenolic compounds

The total flavonoids content in fruits of *Rosa rugosa* were 1.5–1.7 times higher than in the fruit of *Rosa canina* (Figure 4). The ripening stage had significant influence on flavonoids content in rose hips, but the correlation between the levels of flavonoids and mature of fruits was not established. The maximal content was in half-ripe fruits, and the unripe and fully ripe fruits did not differ significant. The content of flavonoids in rose hips collected from various locations was different. The higher level was in fruits from Curonian Spit.

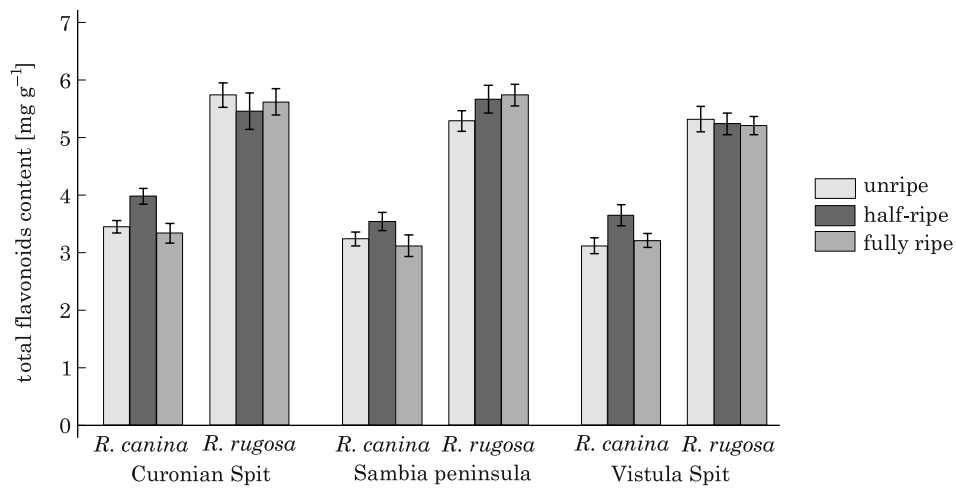

Fig. 4. Total flavonoids content in fruits of *Rosa canina* and *Rosa rugosa* collected at different ripening stages and from three growth locations

The total tannins content in the fruit of *Rosa canina* was significantly higher than in the fruit of *Rosa rugosa* (Figure 5). On average, the difference was about 37% (3.23 and 2.03 mg g^{-1} for *Rosa canina* and *Rosa rugosa* respectively, Table appx. 1). In the process of ripening, total tannins con-

tent increased and reached a maximum in ripe fruits. According to the results of 3-factorial ANOVA the location has also significant effect on tannins accumulation in rose hips (Table appx. 1). The higher levels were in fruits from Curonian Spit and Sambia peninsula.

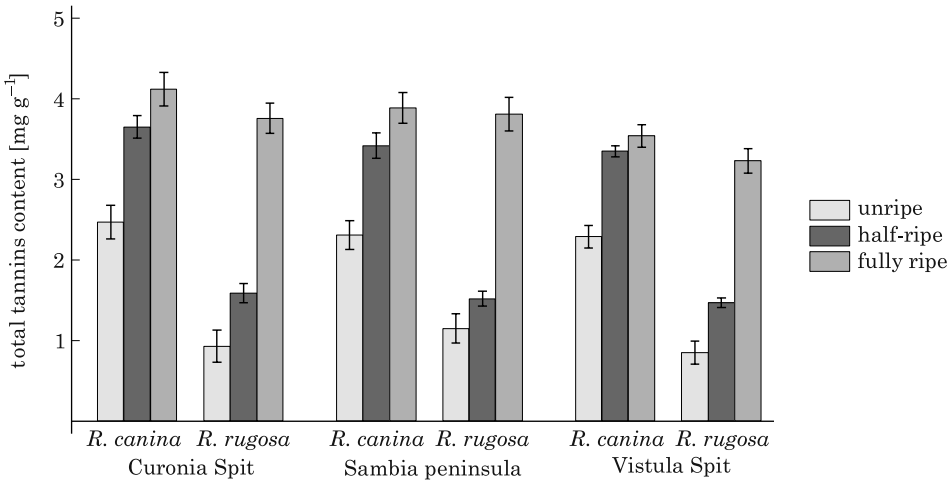

Fig. 5. Total tannins content in fruits of *Rosa canina* and *Rosa rugosa* collected at different ripening stages and from three growth locations

The fruits of *Rosa rugosa* characterized also by higher total phenolic compounds content, especially ripe fruits. For half-ripe and unripe fruits the difference between species was not so great (Figure 6). However, for both species the total phenolics content in rose hips increased during their

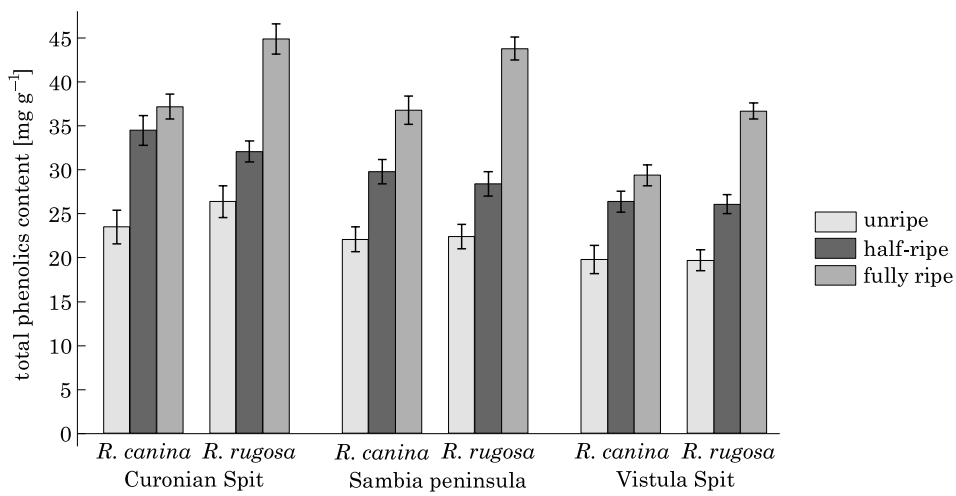

Fig. 6. Total phenolics content in fruits of *Rosa canina* and *Rosa rugosa* collected at different ripening stages and from three growth locations

ripening. The polyphenols content was different in fruits collected from various growth locations. The highest level was in rose hips from Curonian Spit, medium – from Sambia peninsula, and lowest – from Vistula Spit (Table appx. 1).

Total water-soluble antioxidants content and antioxidant capacity

Total water-soluble antioxidants were also varying significantly between the species (Table 1, Table appx. 2). This value was significantly higher (up to 2 times) for the fruit of *Rosa rugosa* ($p \leq 0.05$). The hips of this species may probably have higher total water-soluble antioxidants due to the higher content of ascorbic acid ($r = 0.898$, $p \leq 0.05$) and flavonoids ($r = 0.955$, $p \leq 0.05$,

Table 1

Antioxidant properties of rose hips collected from different location on the Baltic Sea coast (2016 year)

Species	Maturity	Total water-soluble antioxidants content [mg QE g^{-1}]	Total antioxidant capacity [µmol TE g^{-1}]		
			DPPH	ABTS	FRAP
Curonian Spit					
Rosa canina L.	unripe	23.4±1.7	114.1±3.2	298.6±3.6	512.1±5.8
	half ripe	21.3±2.1	126.3±4.6	329.2±6.4	531.1±8.8
	fully ripe	22.0±1.2	127.6±5.4	369.4±4.2	574.1±4.8
Rosa rugosa Thunb.	unripe	41.9±2.0	311.4±9.3	471.6±12.2	621.4±7.2
	half ripe	42.6±2.4	325.4±10.8	568.4±9.7	674.5±11.8
	fully ripe	45.5±3.3	356.3±11.7	721.7±13.6	689.1±9.2
Sambia peninsula					
Rosa canina L.	unripe	25.6±1.4	124.1±4.1	275.4±4.2	498.7±6.4
	half ripe	19.8±1.9	128.4±5.2	314.4±9.6	568.1±10.7
	fully ripe	21.4±1.4	132.2±3.1	344.1±3.6	589.7±5.4
Rosa rugosa Thunb.	unripe	45.8±2.2	325.6±8.4	487.9±10.8	610.3±8.4
	half ripe	44.5±1.8	331.2±9.7	574.1±10.1	665.2±10.7
	fully ripe	47.8±2.1	342.9±4.9	703.7±10.6	674.4±6.7
Vistula Spit					
Rosa canina L.	unripe	19.7±1.1	108.2±2.9	304.6±3.8	468.9±5.4
	half ripe	17.9±1.5	119.7±4.1	297.4±5.6	512.3±7.3
	fully ripe	19.8±0.7	116.3±4.3	312.7±2.8	541.4±3.8
Rosa rugosa Thunb.	unripe	38.7±1.7	298.7±7.4	467.2±12.6	594.2±6.4
	half ripe	36.4±1.6	319.4±8.6	542.3±6.8	632.4±9.5
	fully ripe	42.1±2.4	312.4±8.6	679.8±11.8	634.6±7.2

Table 2
Correlation matrix with the Pearson coefficient values for the bioactive compounds and antioxidants in rose hips

	AsA	Car	TTC	TFC	TPC	TWAOX	DPPH	ABTS	FRAP
AsA	1,000								
Car	0,414	1,000							
TTC	-0,188	0,775*	1,000						
TFC	0,881*	0,078	-0,484*	1,000					
TPC	0,499*	0,899*	0,706*	0,223	1,000				
TWAOX	0,898*	0,116	-0,458	0,955*	0,242	1,000			
DPPH	0,923*	0,135	-0,463	0,977*	0,248	0,978*	1,000		
ABTS	0,973*	0,475*	-0,114	0,877*	0,547*	0,902*	0,918*	1,000	
FRAP	0,936*	0,421	-0,148	0,856*	0,563*	0,859*	0,894*	0,908*	1,000

*Correlation is significant at the 0.05 level (2-tailed), $p < 0.05$; AsA – ascorbic acid, Car – carotenoids, TTC – total tannins content, TFC – total flavonoids content, TPC – total phenolics content, TWAOX – total water-soluble antioxidants content, DPPH, ABTS, FRAP – antioxidant capacity measured by DPPH, ABTS and FRAP assays respectively

Table 2). The estimation of total antioxidant capacity using DPPH, ABTS, and FRAP assays also showed a higher antioxidant value of rose hips of *Rosa rugosa.* At the same time, the results obtained with various assays dispersed. Thus, by using DPPH assay the total antioxidant capacity of rose hips *Rosa rugosa* was almost 3 times higher, by using ABTS assay 2 times higher, and by using FRAP assay only 1.2 times higher (Table 1, Table appx. 2). The higher value of total water-soluble antioxidants content was determined in unripe and fully ripe fruits. Generally, the antioxidant capacity of rose hips increased in process of ripening. The higher values of total antioxidant capacity were identified in ripe fruits compared with unripe and half-ripe, excluding antioxidant capacity defined by using DPPH assay. In this case there was no significant difference between half-ripe and fully ripe rose hips (Table appx. 2). As well as total water-soluble antioxidants content and antioxidant capacity were lower in rose hips collected from Vistula Spit and higher in fruits from Curonian Spit.

Discussion

In the present study, species-specific accumulation of ascorbic acid was investigated. It was shown that the fruits of *Rosa rugosa* had significant more Vitamin C as the fruits of *Rosa canina*. Species-specific accumulation of ascorbic acid was also demonstrated in the study of KAZAK et al. (2009). The authors proved that Vitamin C content in *Rosa canina* L. was

1.3–4.0 times higher than in *Rosa damascene* Mill. depending on the studied part of the plant (fruit, fruit flesh or seed). Research by CZYŻOWSKA et al. (2015) also proved that raw materials from *Rosa rugosa* hips had higher content of ascorbic acid in comparison with *Rosa canina* plants.

Rose hips are a good source of carotenoids and can be used as a raw material in production of health-promoting products. Typical carotenoids in rose hips are mainly lycopene, lutein, and β-carotene (BARROS et al. 2011). Some authors qualified high amount of rubixanthin in rose hips (AL-YAFEAI et al. 2018b). But in study by AL-YAFEAI et al. (2018b) the content and variety of carotenoids were strong depended on rose species. In our study was also demonstrated that fruits of *Rosa rugosa* had more carotenoids compared to *Rosa canina*.

The value of rose hips as being the source of biologically active compounds is also in that the stability of ascorbic acid in the fruit is sustained because the fruit contains phenolic compounds (anthocyanins, tannins, leucoanthocyanins and flavonols) with different chemical structure but yet similar biological action (CARR and VISSERS 2013). In work (STĂNILĂ et al. 2015) big variety of phenolic compounds in the ripe fruit of *Rosa canina* var. Lutetiana & flexibilis was shown. The authors identified up to 19 individual compounds out of the groups of anthocyanins, flavonols, and their glycosides, and phenolic acids. In the present study was shown that the total content of flavonoids and polyphenols was higher in fruits of *Rosa rugosa*, but the total content of tannins was opposite higher in *Rosa canina*. In work (KOCZKA et al. 2018) the concentration of TPC was obtained in a decreasing order of *R. spinosissima* > *R. canina* > *R. rugosa* > *R. gallica*.

Nowadays, there are many very different methods for determining antioxidant activity in plant extracts. The amperometric detection offers a number of advantages. These include a low detection limit, high selectivity (determined are only those compounds whose molecules can be oxidized, while other compounds are not determined, even if in high concentrations), a small volume of the electrochemical cell (0.1–5 µL), and easy servicing (YASHIN 2008). However, as shown in the study of CZYŻOWSKA et al. (2015), when comparing antioxidant activity of wines from R. *canina* and R. *rugosa*, the measuring method should be taken into account. Thus, when using DMPD method, the wine from R. *rugosa* showed higher antioxidant activity, whereas with ABTS method it was higher in the wine from R. *canina*, and there were no significant differences when DPPH method was used. Measuring antioxidant activity in the leaves of different species with DPPH method also revealed no difference between *Rosa rugosa* and *Rosa canina* (NOWAK and GAWLIK-DZIKI 2007).

During the ripening the amount of some compounds decreases, while the amount of others – increases. Although the majority of authors still noted that the level of biologically active compounds increases with the ripening of rose hips. Thus, in the study of NOJAVAN et al. (2008), Vitamin C content in the rose hips of *Rosa canina* was found to be increasing with ripening. And the study of ADAMCZAK et al. (2012) proved that the fully ripe fruit had the maximum content of anthocyanins and flavonols. According to the work by AL-YAFEAI et al. (2018) increasing in total carotenoids content due to the accumulations of (all-E)-*β*-carotene, (all-E)-rubixanthin and (all-E)-lycopene. The synthesis of these secondary metabolites in plant changes according to changes in enzyme activity. Enzyme activities are influenced by many factors including stage of growth and development of the plant. Depending on the enzyme activities can also change the content of biological active compounds in the pseudofruits of *Rosa* species (ELMASTAŞ et al. 2017).

Secondary metabolites, including many biologically active components and antioxidants, are sensitive components of plants, whose levels in a given plant is directly related to the state of environment (pollution of the soil and/or air with xenobiotics) and ecological conditions that determine the growth and development of plants (temperature, light, humidity). This topic is especially relevant for coastal areas, due to climatic features and specific vegetation period of plants. The climate of Kaliningrad region is temperate, marine transitional to continental. The summer is relatively cold and the July mean temperature ranges from +17 to +18°C. The winter is mild. The January mean temperature is -2–-4°C. The average annual precipitation is 750 mm, ranging from 400 to 1100 mm, depending on the prevalence of either continental or marine air masses (FEDOROV et al. 2016). In our study was shown that growth location has significant influence on accumulation of all biological active compounds, with the exception of carotenoids. The main trend was a decrease in the level of biologically active compounds and antioxidants in rose hips, in the direction from the Curonian Spit to the Vistula Spit.

Based on the content of ascorbic acid, carotenoids, flavonoids, tannins, polyphenols, water-soluble antioxidants, and antioxidant capacity of rose hips the hierarchical cluster analysis was performed (Figure 7). The results of this analysis showed that the main factor determining the differences in accumulation of biologically active compounds is the specie of rose followed by ripening stage and growth location.

A lot of studies have reported high biological active compounds content and antioxidant capacities of natural plant sources. As correctly pointed

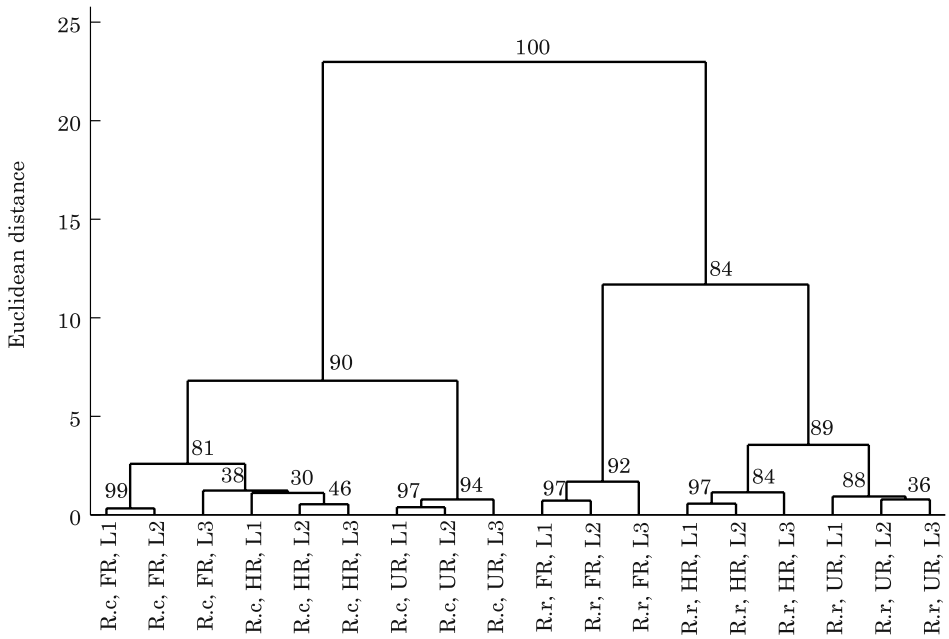

Fig. 7. Dendrogram for the classification of *Rosa* L. fruits collected at different ripening stages and from different locations by Ward's method with respect to the content of studied biological active compounds and antioxidant capacity. Figures at the nodes indicate bootstrap support values after 1000 replicates. R.c. – *Rosa canina*, R.r. – *Rosa rugosa*, FR – fully ripe, HR – half-ripe, UR – unripe, L1 – Curonian Spit, L2 – Sambia peninsula, L3 – Vistula Spit

out in the article of Dr. PETAL (2017) "While consumers are willing to pay exorbitant price for dietary supplements, a natural source of multi-nutrients going to waste is paradoxical". Rose hips are valuable plant resource, containing various biologically active compounds. In our study was established the content of some phytonutrients depending on species and ripening stage of rose hips. *Rosa rugosa* showed higher nutritional value than *Rosa canina* according to the content of vitamin C, the total amount of flavonoids and phenolics, water-soluble antioxidants and antioxidant activity of the rose hips. At the same time, the level of biologically active compounds, especially of ascorbic acid, carotenoids, tannins, and total phenolic compounds, in the fruits of both species increased during the ripening and reached the maximum in fully ripe rose hips. It was also exposed that the content of biological active compounds strongly depends on the place of plant growth and this factor should be taken into account by the collection of rose hips for example for the production of health-promoting products. Thus, in this study was shown high nutritional value of rose hips, especially of the species *Rosa rugosa* Thunb. The results should be

taken into account when deciding on the possible limitation of the spread of this species on the Baltic Sea coast, as well by the including the rose hips in the daily diet or designing on their basis functional foods.

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Appendix

Table appx. 1

Results of 3-factorial ANOVA for bioactive compounds

		AsA	Car	TFC	TTC	TPC
Main effects						
Species (Sp)	*R. canina*	8.97*b*	0.61*b*	3.41*b*	3.23*a*	28.8*b*
	R. rugosa	32.3*a*	0.64*a*	5.48*a*	2.03*b*	31.7*a*
Ripening stage (RS)	unripe	14.3*c*	0.35*c*	4.37*b*	1.67*c*	22.3*c*
	half ripe	21.7*b*	0.52*b*	4.60*a*	2.50*b*	29.6*b*
	fully ripe	25.8*a*	0.94*a*	4.38*b*	3.73*a*	38.1*a*
Location (L)	Curonian Spit	21.4*a*	0.61*a*	4.61*a*	2.75*a*	33.1*a*
	Sambia peninsula	21.7*a*	0.63*a*	4.44*b*	2.68*a*	30.6*b*
	Vistula Spit	18.8*b*	0.62*a*	4.23*b*	2.43*b*	26.4*c*
Significance	Sp	*	*	*	*	*
	RS	*	*	*	*	*
	L	*	ns	*	*	*
	Sp*RS	*	*	*	*	*
	Sp*L	*	*	ns	ns	ns
	RS*L	*	*	*	*	*
	Sp*RS*L	ns	*	*	ns	ns

Data was evaluated via three-way ANOVA, factors: species, ripening stage and location, $\alpha = 0.05$, followed by Tukey HSD test (mean, $n = 6$). Identical letters indicate that values do not differ significantly. Asterisks indicate significantly influential factors. AsA – ascorbic acid, Car – carotenoids, TTC – total tannins content, TFC – total flavonoids content, TPC – total phenolics content

Table appx. 2

Results of 3-factorial ANOVA for antioxidants

		AOX	DPPH	ABTS	FRAP
Main effects					
Species (Sp)	*R. canina*	21.2*b*	121.9*b*	316.2*b*	532.9*b*
	R. rugosa	42.8*a*	324.8*a*	579.6*a*	644.0*a*
Ripening stage (RS)	unripe	32.5*a*	231.3*b*	384.2*c*	550.9*b*
	half ripe	30.4*b*	225.1*ab*	437.6*b*	597.3*a*
	fully ripe	33.1*a*	231.7*a*	521.9*a*	617.2*a*
Location (L)	Curonian Spit	32.8*a*	226.9*a*	459.8*a*	600.4*a*
	Sambia peninsula	34.2*a*	230.7*a*	449.9*ab*	601.1*a*
	Vistula Spit	29.1*b*	212.5*b*	434.0*b*	563.9*b*
Significance	Sp	*	*	*	*
	RS	*	*	*	*
	L	*	*	*	*
	Sp*RS	*	*	*	*
	Sp*L	*	*	*	*
	RS*L	ns	*	*	*
	Sp*RS*L	ns	*	*	*

Data was evaluated via three-way ANOVA, factors: species, ripening stage and location, $\alpha = 0.05$, followed by Tukey HSD test (mean, $n = 6$). Identical letters indicate that values do not differ significantly. Asterisks indicate significantly influential factors. AOX – total water-soluble antioxidants content, DPPH, ABTS, FRAP – antioxidant capacity measured by DPPH, ABTS and FRAP assays respectively