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Carotenoids, Polyphenols, and Ascorbic Acid in Organic Rosehips (*Rosa spp.*) Cultivated in Lithuania

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Abstract: Carotenoids, polyphenols, and ascorbic acid are valuable and important biologically active compounds that contribute to the health benefits of different foods, and rosehips are known for their high biologically active substance contents. The aim of this research is to identify and compare the contents of carotenoids, polyphenols, and ascorbic acid in the flesh and seeds of different rosehip species grown organically. A field experiment was conducted during the period 2017–2018 on an organic farm in Lithuania. Rose fruits were collected at full ripening in September. The quantitative and qualitative determinations of polyphenols and carotenoids in rosehips flesh and seeds were conducted by HPLC methods. The following polyphenolic compounds were identified: five different flavonoids (rutin, kaempferol-3-*O*-glucoside, luteolin, quercetin, and quercetin-3-*O*-glucoside) and five phenolic acids (gallic, chlorogenic, caffeic, *p*-coumaric, and ferulic) from different rose fruit fleshes and seeds species. In addition, six carotenoids (β-carotene, α-carotene, lutein, zeaxanthin, *cis*-lycopene, and *trans*-lycopene) in rosehip flesh and five different carotenoids in rosehip seeds (α-carotene, lutein, zeaxanthin, *cis*-lycopene, and *trans*-lycopene) were identified. Overall, the results of this study demonstrate that the highest content of total phenolic acid is in rosehip seeds, while higher concentrations of carotenoids, flavonoids, and ascorbic acid are present in rosehip flesh.

Keywords: polyphenols; carotenoid; seeds; flesh; rosehip

1. Introduction

In recent decades, the hips of *Rosa* species have gained increasing interest due to their high amounts of biologically active compounds. These compounds have potential effects on human health by reducing the risk of diseases such as cancers and cardiovascular diseases [1]. Bioactive compounds like ascorbic acid (125–2650 mg 100 g^{-1} fresh fruits) [2,3], phenolic compounds (31.08–52.94 mg gallic acid equivalent (GAE) g^{-1}) [4], tocopherols (7–15 mg 100 g^{-1}) [5], carotenoids (30–760 g kg^{-1} dry weight (DW)) [6], linoleic and α-linolenic acids (4.85–54.75%) [7] are particularly abundant in the rosehip fruits' flesh and peel.

Rosehips fruits have long been used for food and medicinal purposes. They are used in many drinks and foodstuffs [8]. Rosehips are used in medicine as an herbal remedy for the treatment of various ailments (flu, colds, inflammations, chronic pain, etc.) and skin care [8,9].

The fruits of different rose species are a rich source of ascorbic acid. According to the literature, the fresh rosehip has the highest content of this vitamin between berries and fruits [10].

Carotenoids, lutein, and zeaxanthin are pigments produced by plants that give yellow, orange, and red pigments. Both are potent antioxidants that offer a range of human health benefits [11]. It has been found that antioxidants protect against cellular damage due to their ability to quench oxygen-derived free radicals [12]. Studies in the human body show that these important carotenoids have antioxidant abilities that are essential nutrients for human eyes. In the eye, lutein and zeaxanthin are present as macular pigments and may protect the macula and photoreceptor outer segments throughout the retina from oxidative stress and play a role in an antioxidant cascade that safely disarms the energy of reactive oxygen species [13]. Rosehip contains both important carotenoids and patients with macular degeneration have achieved visual improvement with rosehip seed and shell products [14].

Phenolic compounds in food products of plant origin are secondary plant metabolites that protect plants from UV light and infections or act as attractants for pollinators [15,16]. Their antioxidant activity as radical scavengers, bioavailability, others beneficial roles of polyphenols are already known [17,18]. In previous studies, the dominated phenolic acids (ferulic acid, gallic, chlorogenic, *p*-coumaric, *t*-caffeic, and sinapic acids), flavanols, flavanones, proanthocyanidin aglycones, glycosides [4,19,20], and carotenoids [1] in rose fruits are presented, but there is a lack of researchers data's of phenolic and carotenoid compounds when *Rosa* species have been grown in organic farming.

Moreover, some biologically active compounds are located in rosehip seeds, and are eliminated as waste. Therefore, the purpose of this research was to investigate and compare the contents of carotenoids, polyphenols, and ascorbic acid in the flesh and seeds of different rosehip species grown organically.

2. Materials and Methods

2.1. Field Experiment

A one-factor field experiment with rosehips species, *Rosa rugosa* (RR), *Rosa rugosa* 'Rubra' (RRR), *Rosa rugosa* 'Alba' (RRA), *Rosa canina* (RC), and *Rosa villosa* (RV) (Figure 1) was conducted during the period 2017–2018 on an organic farm (certificate No. SER-K-17-01478) in Pakruojis District, Lithuania (56°10'29.0" N 23°49'02.6" E). The soil in the experimental field was characterized by close to neutral acidity (pH_{KCl} = 6.14–6.85), medium potassium status (K_2O = 83.2–154.8 mg kg^{-1}), and medium phosphorus status (P_2O_5 = 122.6–137.1 mg kg^{-1}), and the total nitrogen content was 2.67%. The experimental plot was 2000 m^2, and all plots were prepared randomly with four replicates. The distance between the rows was 4 m, and the distance between the rosehip shrubs was 2 m. The plants were 9 years old.

2.2. Preparation of Rosehips Samples

Rosehips were picked by hand in September at full ripening. A total of 1 kg of fruits was picked from each species, from four plants, from each replication. For investigation the stem was removed from the rosehips, and then was cut in half and the seeds separated. The rosehips flesh and seeds were frozen at −35 °C and lyophilized for 48 h in Freeze-Drying Plant Sublimator (ZIRBUS GmbH, Bad Grund, Germany). The lyophilized fruit flesh and seeds were milled and stored in sealed containers at 5 °C in the dark until investigation.

2.3. Soil Agrochemical Analyses

The soil samples were air-dried, and the remains of roots, other plant parts, and small stones were removed. Soil samples were crushed and sieved with a 1 mm sieve. The following parameters were investigated: available potassium, available phosphorus, total nitrogen, and pH_{KCl}. Available potassium (mg kg^{-1}) and phosphorus (mg kg^{-1}) were extracted with ammonium-lactate according to the Egner–Riehm–Domingo method. Total nitrogen concentration (mg kg^{-1}) was established by the Kjeldahl method. Soil pH_{KCl} was determined by the potentiometric method in 1N KCl extract.

Genotype		Colour at full ripening stage	Width of fruit, mm	Length of fruit, mm
Rosa Rugosa		More than 90% of the surface is red	17.72–25.83	17.23–26.07
***Rosa Rugosa* 'Rubra'**		**More than 90% of the surface is red.**	**22.23–25.98**	**20.94–26.02**
***Rosa rugosa* 'Alba'**		More than 90% of the surface is red	20.23–25.89	19.98–26.14
Rosa Villosa		**More than 90% of the surface is red**	**15.67–24.34**	**20.11–25.05**
Rosa Canina		More than 90% of the surface is red	9.66–11.04	15.01–18.05

Figure 1. Fruits of different organic *Rosa* species.

2.4. Carotenoids Determination

The carotenoids content was determined by the method that was described by Hallmann [21], with some modifications. A weighed amount of freeze-dried rose fruits flesh and seeds sample (100 mg) was put into a plastic test tube, and then 1 mL hexane (Sigma Aldrich, Warsaw, Poland) and 2 mg of magnesium carbonate (Mg_2CO_3) were mixed thoroughly by vortex and incubated in an ultrasonic cold ultrasonic bath (15 min at 0 °C). Then, the samples were centrifuged at the speed of 6000× *g* rpm (10 min at 0 °C), and the gradient flow was applied along with two mobile phases: acetonitrile with methanol (90:10) and methanol with ethyl acetate (68:32) with a flow rate of 1 mL min^{-1}. From the test tube, 1 mL supernatant was collected and was re-centrifuged at 12,000× *g* rpm. Then, 900 µL of supernatant were injected for high-performance liquid chromatography (HPLC) (Shimadzu, USA Manufacturing Inc., Canby, OR, USA). To determine the carotenoids, the HPLC set-up consisted of two LC-20AD pumps, a CMB-20A system controller, an SIL-20AC auto sampler, an ultraviolet–visible SPD-20AV detector, a CTD-20AC oven, and a Phenomenex Max 80-Å RP column (250 × 4.60 mm)

from Shimazu (Shim-Pol, Warsaw, Poland). The wavelength used was 450–471 nm. Lycopene (Sigma Aldrich, Warsaw, Poland) and β-carotene (Fluka, Warsaw, Poland), with purities of 99%, were used as the external standards (Figure S1). Each measurement was performed in triplicate.

2.5. Polyphenols Determination

Polyphenols were determined with a method that was described by Hallmann [21], by using the HPLC equipment described above. First, 100 mg of freeze-dried rosehip flesh and seed sample were put into a plastic test tube, and then 5 mL methanol were mixed thoroughly by the vortex and incubated in an ultrasonic bath (15 min at 30 °C). Then, the samples were centrifuged at the speed of 6000× *g* rpm (10 min at 0 °C). Next, 1 mL of extract was obtained from the test tube and was collected and re-centrifuged at the speed of 12,000× *g* rpm. An amount of 500 µL extract was taken for the HPLC vials and analyzed. A HPLC column (Phenomenex, Fusion-80A, C-18, practical shape 4 µm, 250 × 4.6 mm, Shim-Pol, Warsaw, Poland) was used for the analysis of polyphenols. The gradient flow had two mobile phases: acetonitrile water (55%) and deionized water (10%) at pH 3.00. The time of the analysis was 38 min, the flow rate was 1 mL min^{-1}, and the wavelength was 280–340 nm. Phenolic compounds were identified based on Fluka and Sigma Aldrich (Warsaw, Poland) external standards (Figures S2 and S3) with a purity of 99%. All the described chemical composition analyses were performed in 3 replications.

2.6. Ascorbic Acid Determination

Ascorbic acid was measured by titration with a 2,6-dichlorophenolindophenol sodium salt solution and chloroform was used for intensely colored extracts [22].

2.7. Statistical Analysis

Statistical analyses on the bioactive compounds of rosehips flesh and seeds were performed with Microsoft® Excel® 2016 MSO and verified by statistical software STATISTICA (Statistica 10, StatSoft, Inc., Tulsa, OK, USA, 2010) package. The credibility of the results was evaluated by one-way analysis of variance (ANOVA). Statistical reliability was established by Fisher's least significant difference (LSD) test. Differences among results were considered to be significant if $p < 0.05$. In addition, the correlation analysis was carried out to establish the strength and nature of the correlation among the bioactive compounds (total polyphenols content, total flavonoids content, total phenolic acids content, total carotenoids content, and ascorbic acid content). Hierarchical cluster analysis was performed to categorize the rosehip flesh and seed samples based on their bioactive compounds (carotenoids, polyphenols, and ascorbic acid) content (XLSTAT Software, version 2016, Addinsoft, New York, NY, USA, 2016).

3. Results and Discussion

3.1. Carotenoids Composition

According to the literature, rosehips have a high level of carotenoids compared to other fruits [23]. Our study showed that in the flesh of organic rosehips lutein and zeaxanthin were composed from 12.89–20.53%, β-carotene 45.56–70.34%, α-carotene 6.97–13.51%, and total lycopene 9.29–24.68% of the total amount of carotenoids. Carotene corresponded from 54.79% to 77.31% of total carotenoids in rosehips flesh (Table 1).

Table 1. Carotenoid composition and content (mg 100 g^{-1} dry weight (DW)) of different organic rosehip species flesh and seeds.

Rosehip Species	Carotenoid Composition, mg 100 g^{-1} DW						
	Total Carotenoid	β-Carotene	α-Carotene	Lutein	Zeaxanthin	*Cis*-Lycopene	*Trans*-Lycopene
				In rosehips flesh			
RRA	25.29 ± 0.54 b	16.48 ± 0.42 b	2.32 ± 0.11 b	3.87 ± 0.13 b	0.27 ± 0.02 b	0.59 ± 0.02 b	1.76 ± 0.02 b
RRR	16.58 ± 0.58 c	9.40 ± 0.43 d	2.24 ± 0.06 b	2.68 ± 0.17 c	0.25 ± 0.01 c	0.49 ± 0.01 d	1.52 ± 0.05 c
RR	18.07 ± 0.93 c	12.71 ± 1.07 c	1.26 ± 0.11 c	2.68 ± 0.15 c	0.24 ± 0.005 cd	0.25 ± 0.01 e	0.93 ± 0.02 d
RC	8.67 ± 0.93 d	3.95 ± 0.80 e	0.80 ± 0.12 d	1.55 ± 0.08 d	0.23 ± 0.003 d	0.55 ± 0.03 c	1.59 ± 0.09 c
RV	49.51 ± 1.04 a	31.40 ± 0.22 a	6.11 ± 0.35 a	6.06 ± 0.56 a	0.32 ± 0.02 a	1.44 ± 0.03 a	4.18 ± 0.04 a
				In rosehips seeds			
RRA	0.62 ± 0.004 c	-	-	-	0.21 ± 0.004 c	0.13 ± 0.001 a	0.28 ± 0.001 b
RRR	1.19 ± 0.09 a	-	0.20 ± 0.07 a	0.31 ± 0.01 b	0.22 ± 0.006 b	0.14 ± 0.01 a	0.32 ± 0.005 a
RR	0.55 ± 0.07 c	-	-	-	0.23 ± 0.004 a	0.06 ± 0.07 b	0.26 ± 0.004 c
RC	0.58 ± 0.003 c	-	-	-	0.21 ± 0.004 c	0.13 ± 0.001 a	0.24 ± 0.001 d
RV	1.07 ± 0.03 b	-	0.04 ± 0.01 b	0.40 ± 0.02 a	0.22 ± 0.007 b	0.13 ± 0.001 a	0.28 ± 0.007 b

In the same column, different small letters represent significant differences between flesh samples and seed samples species, respectively ($p < 0.05$). RRA: *Rosa rugosa* 'Alba'; RC: *Rosa canina*; RR: *Rosa rugosa*; RRR: *Rosa rugosa* 'Rubra'; RV: *Rosa villosa*.

It is generally known that rosehips are rich in bioactive compounds, such as carotenoids. However, the differences in content of these compounds depend on many factors: genetic variation, maturity of the fruit, degree of ripening, growing, climate, and storage conditions, as well as, extraction and analytical method [24].

The total content of lycopene (*cis*-lycopene + *trans*-lycopene) in organic rosehip flesh ranged from 1.18 mg g^{-1} to 5.62 mg g^{-1} DW, and the significantly highest amount was identified in RV flesh (Table 1). The isomer pattern, with its high relative content of *cis*-isomers of lycopene, was unexpected. Rosehips flesh showed a ratio of *trans*-lycopene to *cis*-lycopene isomers ranging from 74:26 to 79:21 (Table 1).

Other authors [5,23] show much lower amounts of lutein and zeaxanthin, at concentrations of 2.21 mg 100 g^{-1} and 2.00 mg 100 g^{-1}, respectively. Our results showed that the lutein and zeaxanthin amount was significantly higher, namely, 6.38 mg 100 g^{-1} DW in RV species flesh (Table 1).

The orange pigment beta carotene is the most common of various carotenoids in food products, either as a major or minor ingredient [25]. The significant differences in the amount of β-carotene were investigated for all species of organic rosehips. According to literature the carrot (*Daucus carrota* L.) has some of the greatest levels of beta carotene, and the concentrations of this carotenoid in carrot may fluctuate from 3.2 mg 100 g^{-1} to 6.1 mg 100 g^{-1} fresh weight (FW) [26].

However, rosehip flesh can have higher β-carotene concentrations than that of carrot, and the values range from 3.95 mg 100 g^{-1} to 31.40 mg 100 g^{-1} DW (Table 1). Our data showed that the amount of β-carotene in RC species was eight times lower, while the amount detected in the flesh of RV species was significantly higher (31.40 mg 100 g $^{-1}$ DW).

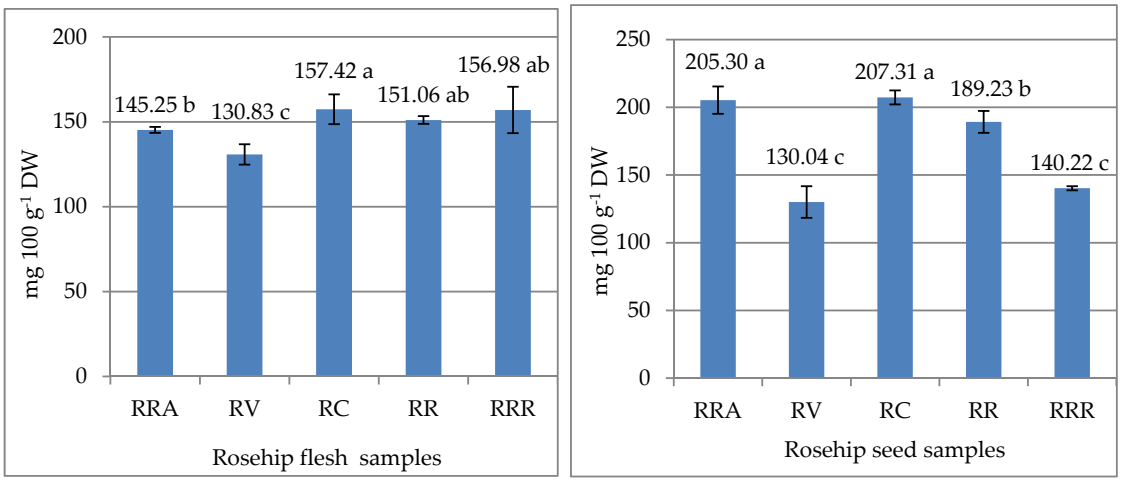
The quantitative and qualitative composition of carotenoids in organic rose fruit flesh and seeds varied dependently. β-carotene, α-carotene, lutein, zeaxanthin and *cis*- and *trans*-lycopene were detected in flesh, while β-carotene was not detected in seeds. In addition, rosehip seeds revealed a lower carotenoid content than did rosehip flesh, and α-carotene and lutein were obtained only in the RRR and RV species (0.20 and 0.31, 0.04 and 0.40 mg 100 g^{-1} DW, respectively) (Table 1).

Al-Yafeai et al. [1] also confirmed that carotenoid contents varied between rosehips species. These authors found that *R. rugosa* hips contained (all-E)-violaxanthin and (5′Z)-rubixanthin (gazaniaxanthin) in contrast to *R. canina* hips. Moreover, *R. rugosa* also contained a higher content of (all-E)-β-cryptoxanthin (1.2 mg 100 g^{-1}) compared with *R. canina* hips (0.1 mg 100 g^{-1}). However, *R. canina* hips contained a higher content of (all-E)-α-cryptoxanthin (1.1 mg 100 g^{-1}), compared to *R.* rugosa hips (0.01 mg 100 g^{-1}). A study conducted on five *Rosa* species (*R. canina*, *R. moschata*, *R. damascena*, *R. webbiana*, and *R. hemisphaerica*) reported concentrations of total carotenoid 3.03 mg 100 g^{-1} (*R. moschata*) and 20.21 mg g^{-1} fresh weight (FW) (*R. damascena*). Total lycopene and β-carotene

contents ranged between 4.00 μg g^{-1} (*R. webbiana*) and 26.80 μg g^{-1} FW (*R. damascena*) and between 1.02 μg g^{-1} (*R. moschata*) and 8.70 μg g^{-1} FW (*R. canina*), respectively. According the authors of this study, the variations of carotenoids level among the species of Rosa fruits could be attributed to species, climatic conditions, degree of maturation, and harvest time [27].

3.2. Total Polyphenols Content

The content of total polyphenols in organic rosehip species ranged from 130.83 mg 100 g^{-1} (RV) to 157.42 mg 100 g^{-1} DW (RC) for flesh (Figure 2a) and from 130.04 mg 100 g^{-1} (RV) to 207.31 mg 100 g^{-1} DW (RC) for seeds (Figure 2b). The RC, RRR, and RR flesh exhibited the highest contents of total polyphenols (157.42, 156.98, and 151.06 mg 100 g^{-1} DW, respectively), with no significant difference between them. The RV flesh had the significantly lowest content of total polyphenols. In the seeds, RRA and RC contained the significantly highest content of total polyphenols, while RV and RRR showed the significantly lowest content of these compounds.



(**a**) (**b**)

Figure 2. Total polyphenols content (mg 100 g^{-1} DW) of different organic rosehip flesh (**a**) and seed (**b**) samples species. Different small letters represent significant differences between flesh samples and seed samples species, respectively ($p < 0.05$). RRA: *Rosa rugosa* 'Alba'; RC: *Rosa canina*; RR: *Rosa rugosa*; RRR: *Rosa rugosa* 'Rubra'; RV: *Rosa villosa*.

In contrast to our data, Barros et al. [28] showed that the rosehip seed had a lower content of total polyphenols compared to the flesh. Koczka et al. [29] found that the contents of polyphenols in rosehips flesh varied from 150.8 mg 100 g^{-1} to 299.2 mg 100 g^{-1} DW. These results coincide with our results. In this study, *R. spinosissima* contained the significantly highest content of phenolic, while R. *canina* and R. *rugosa* showed the significantly lowest content of these compounds. The total phenols content of the studied seed samples was found to be lower than the seed studied by Ilyasoglu [30] (255.40 mg 100 g^{-1} DW).

3.3. Phenolic Acids Composition

Five phenolic acids such as chlorogenic, gallic, caffeic, *p*-coumaric, and ferulic acid were found in the flesh and seeds of all the investigated organic rosehip species (Table 2). The statistically verified high content of total phenolic acid was identified in RRA seeds and in RC species flesh at concentrations of 177.03 mg 100 g^{-1} and 121.81 mg 100 g^{-1} DW, respectively. The rosehip flesh showed a higher *p*-coumaric content, while gallic acid dominated in the seeds. Research has shown that the variation of total phenolic acids was significantly influenced by the genetic characteristics of the species, and essential differences in the percentages of acids in the flesh were identified among all the tested species.

Table 2. Phenolic acid (mg 100 g^{-1} DW) of different organic rosehip flesh and seed species.

Rosehip Species	Total Phenolic Acids	Phenolic Acids, mg 100 g^{-1} DW				
		Gallic	Chlorogenic	Caffeic	*p*-Coumaric	Ferulic
In rosehip flesh						
RRA	107.62 ±1.34 c	15.80 ± 0.23 c	8.02 ± 0.18 d	9.24 ± 0.42 c	55.09 ± 0.96 a	19.46 ± 0.13 b
RC	121.81 ± 1.91 a	22.67 ± 0.65 b	9.80 ± 0.11 cd	22.08 ± 2.86 ab	48.22 ± 6.96 a	19.03 ± 0.41 b
RR	116.83 ± 2.67 b	36.77 ± 0.04 a	14.95 ± 0.26 b	16.86 ± 2.90 b	25.38 ± 2.26 b	22.87 ± 0.42 a
RRR	119.61 ± 2.67 b	36.99 ± 2.37 a	21.01 ± 2.87 a	24.54 ± 7.96 a	25.55 ± 1.10 b	11.51 ± 0.29 c
RV	89.23 ± 1.65 d	36.61 ± 1.61 a	11.08 ± 0.22 c	8.72 ± 0.40 c	21.27 ± 2.56 b	11.55 ± 1.25 b
In rosehip seeds						
RRA	177.03 ± 7.05 a	153.71 ± 6.07 a	3.67 ± 0.03 b	0.99 ± 0.09 c	15.56 ± 0.58 c	3.08 ± 0.45 c
RC	175.12 ± 5.40 a	89.69 ± 0.78 c	45.83 ± 1.97 d	12.73 ± 0.78 a	22.04 ± 2.34 b	4.83 ± 0.57 b
RR	174.17 ± 8.08 a	97.29 ± 3.75 bc	2.02 ± 0.03 a	1.99 ± 0.05 b	64.72 ± 4.61 a	8.15 ± 0.26 a
RRR	114.86 ± 1.65 b	103.94 ± 2.93 b	3.24 ± 0.07 ab	0.67 ± 0.10 c	6.62 ± 0.46 e	0.39 ± 0.01 d
RV	111.59 ± 10.49 b	92.48 ± 10.02 c	6.33 ± 1.12 c	0.79 ± 0.03 c	11.57 ± 1.15 d	0.40 ± 0.01 d

In the same column, different small letters represent significant differences between flesh samples and seed samples species, respectively ($p < 0.05$). RRA: *Rosa rugosa* 'Alba'; RC: *Rosa canina*; RR: *Rosa rugosa*; RRR: *Rosa rugosa* 'Rubra'; RV: *Rosa villosa*.

Nowak [19] reported that the content of gallic acid was 94.3 mg 100 g^{-1} DW in *Rosa rugosa*, 41.0 mg 100 g^{-1} DW in *Rosa villosa*, and 43.5 mg 100 g^{-1} DW in *Rosa canina* rosehip flesh species. The gallic acid levels were 61% higher in *Rosa rugosa*, 11% higher in *Rosa villosa*, and 49% higher in *Rosa canina* compared with our results.

The data demonstrated that the significantly higher concentrations of caffeic (24.54 mg 100 g^{-1} DW) and *p*-coumaric (48.22 mg 100 g^{-1} DW) acids were identified in RRR and RC flesh species, compared with those of Nowak [19]. The causes for these variations may be related to different climatic and environmental conditions (light, temperature, soil nutrients) and the maturity of the fruit, which may influence the accumulation of phenolic compounds [14]. Moreover, some enzymes and genes can also contribute to the biosynthesis and accumulation of these compounds. The activity of these enzymes and genes varies in different genotypes and species [31].

Czyzowska et al. [32] found that the predominant phenolic acids in wine obtained from *R. rugosa* and *R. canina* fruits were gallic acid (144.02 mg L^{-1}) and chlorogenic acid (40.91 mg L^{-1}), respectively. Moreover, wines from *Rosa canina* L. contained higher levels of total polyphenols and flavonoids.

3.4. Flavonoids Composition

Previous studies of flavonoids indicated that these compounds accumulate in epidermal layers and harness damaging ultraviolet B radiation. Rosehip seed oil is rich in flavonoids, and it is used in many countries to protect skin from sunburns [33]. Flavonoids are important for plants, as well. They impact the aroma and color of fruits and flowers and may protect against stress caused by various factors (biotic and abiotic) [34].

Our results showed that more total flavonoids were found in organic rosehips flesh if compared to those with seeds. The highest total flavonoids content was found in RV species flesh (41.59 mg 100 g^{-1} DW) and RC species seeds (32.19 mg 100 g^{-1} DW) (Table 3).

Other studies indicated that rosehips flesh and seeds accumulated a higher number of flavonoids. The total flavonoid level that was observed in different species rosehips ranged from 52 mg 100 g^{-1} to 56 mg 100 g^{-1} in flesh and 52 mg 100 g^{-1} in seeds [33,35].

Rutin was present in the highest concentration among the analyzed flavonoids. Previous studies of this compound indicated that it may control the induction of arteriosclerosis, and the increase in fat in serum. Quercetin is another important compound of rosehip, and it has been reported to have antithrombotic properties, to protect the low-density lipoprotein from oxidation, and to relax the cardiovascular smooth muscles [36]. Depending on the species, the rutin contents in organic rosehip flesh fluctuated from 11.62 mg 100 g^{-1} to 25.54 mg 100 g^{-1} DW, while in seeds, the concentrations of

rutin ranged from 6.86 mg 100 g^{-1} to 20.21 mg 100 g^{-1} DW. The content of quercetin in rosehip flesh ranged from 5.55 mg g^{-1} to 9.77 mg g^{-1} DW, and the significantly highest amount was identified in RC flesh (Table 3).

Table 3. Flavonoids composition and content (mg 100 g^{-1} DW) of different organic rosehip flesh and seeds species.

Rosehip Species	Total Flavonoids	Flavonoids Composition, mg 100 g $^{-1}$ DW				
		Rutin	Kaempferol-3-*O*-Glucoside	Luteolin	Quercetin	Quercetin-3-*O*-Glucoside
			In rosehip flesh			
RRA	37.63 ± 1.01 ab	12.39 ± 0.83 b	11.62 ± 0.34 a	5.81 ± 0.20 b	6.72 ± 0.29 b	1.09 ± 0.16 c
RC	35.61 ± 0.70 b	11.62 ± 0.31 b	4.40 ± 0.71 d	7.46 ± 0.12 a	9.77 ± 0.24 a	2.35 ± 0.12 b
RR	34.23 ± 0.85 b	14.94 ± 0.53 b	7.40 ± 0.46 b	4.97 ± 0.29 c	5.74 ± 0.37 c	1.18 ± 0.19 c
RRR	37.77 ± 3.63 ab	21.23 ± 3.52 a	3.84 ± 0.23 d	2.27 ± 0.10 e	5.55 ± 0.21 c	4.47 ± 0.08 a
RV	41.59 ± 6.61 a	25.54 ± 6.05 a	6.40 ± 0.30 c	4.57 ± 0.04 d	4.77 ± 0.26 d	0.62 ± 0.21 d
			In rosehip seeds			
RRA	28.28 ± 3.09 b	20.21 ±3.21 a	0.96 ± 0.06 b	1.83 ± 0.01 c	1.65 ± 0.04 b	3.63 ± 0.33 b
RC	32.19 ± 0.88 a	19.11 ± 0.81 a	5.29 ± 0.40 a	1.89 ± 0.02 b	2.87 ± 0.39 a	3.02 ± 0.71 b
RR	15.06 ± 0.22 e	6.86 ± 0.22 d	1.03 ± 0.06 b	1.88 ± 0.03 b	2.11 ± 0.05 b	3.19 ± 0.06 b
RRR	25.36 ± 1.27 c	14.75 ± 1.41 b	0.53 ± 0.01 c	2.09 ± 0.03 a	3.08 ± 0.87 a	4.90 ± 0.83 a
RV	18.46 ± 1.97 d	10.59 ± 1.99 c	0.57 ± 0.03 c	1.76 ± 0.02 d	2.09 ± 0.05 b	3.46 ± 0.10 b

In the same column, different small letters represent significant differences between flesh samples and seed samples species, respectively ($p < 0.05$). RRA: *Rosa rugosa* 'Alba'; RC: *Rosa canina*; RR: *Rosa rugosa*; RRR: *Rosa rugosa* 'Rubra'; RV: *Rosa villosa*.

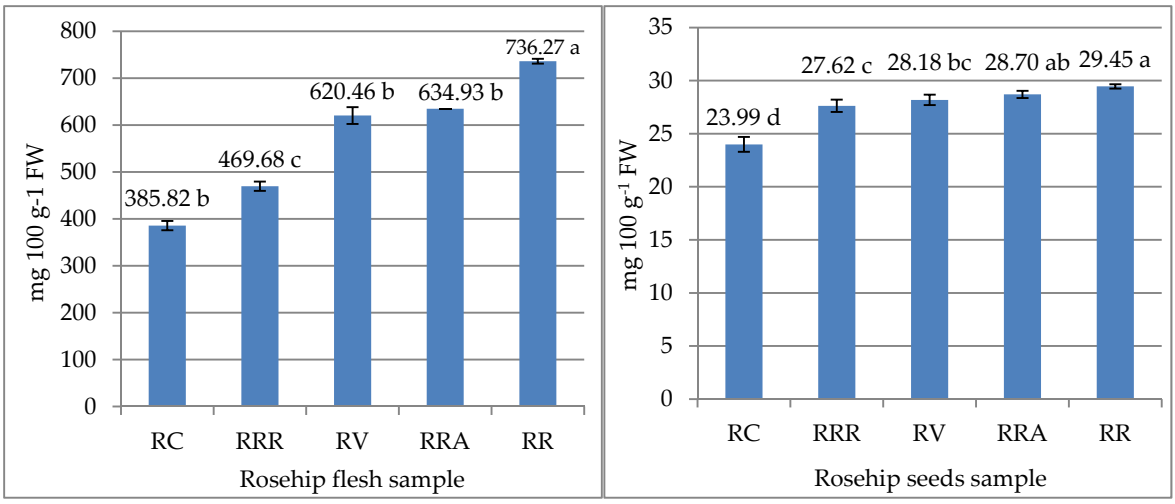
According to the literature, kaempferol-3-*O*-glucoside, also known as astragalin, has anti-atopic, anti-inflammatory, anti-apoptosis, anti-autophagy, and antioxidant properties. Moreover, it has been reported to be a new therapeutic agent for the management of menopausal symptoms [37,38]. Our research showed that the highest kaempferol-3-*O*-glucoside content was found in RRA species flesh (11.62 mg 100 g^{-1} DW) and RC species seeds (5.29 mg 100 g^{-1} DW) (Table 3).

In other studies, Tumbas et al. [39] and Hosni et al. [40] found ellagic acid and quercetin as the main phenolic components of *Rosa canina*. However, other researchers identified catechin and quercetin as the major phenolics in the species, with an absence of ellagic acid or kaempferol [41]. Our results demonstrated that in all tested organic rosehip species, the flesh accumulated a substantially larger amount of rutin, kaempferol-3-*O*-glucoside, luteolin, and quercetin, while more quercetin-3-*O*-glucoside was observed in the seeds.

According to other studies, the flavonoid contents in Rosa fruits depend on environmental and genetic variations. Both genetic and environmental factors participate in the biosynthesis and accumulation of these compounds [4].

3.5. Ascorbic Acid Content

Rosehip fruits are known as medicines since prehistoric times and ascorbic acid is concentrated in the rosehip flesh. The ascorbic acid content of all organic rosehips species ranged in the flesh between 385.82 mg 100 g^{-1} (RC) and 736.27 mg 100 g^{-1} FW (RR) (Figure 3a) and in seeds between 23.99 mg 100 g^{-1} (RC) and 29.45 mg 100 g^{-1} FW (RR) (Figure 3b). In addition, the content of ascorbic acid in RR was highest both in flesh and seeds. Our study showed that contents of ascorbic acid differed greatly between the rose species as well as various parts of fruits (flesh and seeds).



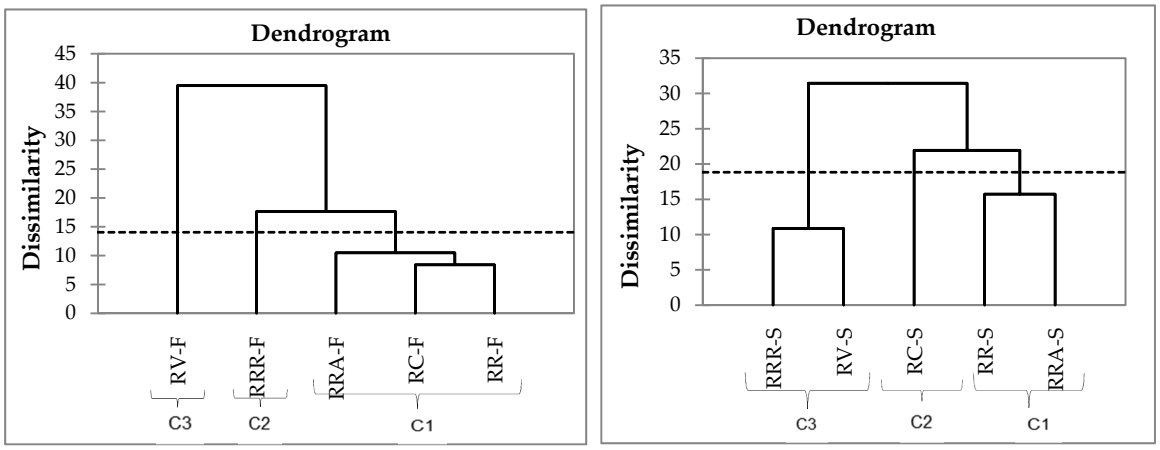
(**a**) (**b**)

Figure 3. Ascorbic acid content (mg 100 g^{-1} fresh weight (FW)) of different organic rosehip flesh (**a**) and seed (**b**) samples species. Different small letters represent significant differences between flesh samples and seed samples species, respectively ($p < 0.05$). RRA: *Rosa rugosa* 'Alba'; RC: *Rosa canina*; RR: *Rosa rugosa*; RRR: Rosa rugosa 'Rubra'; RV: *Rosa villosa*.

The difference in ascorbic acid content of the organic fruits could be caused by the species, variety, ecological factors, and place of growth. Moreover, several researchers pointed that the rosehips, from different agro-climatic regions of Turkey, are a rich source of ascorbic acid and the content ranged from 106 mg 100 g^{-1} to 2712 mg 100 g^{-1} [3].

3.6. Hierarchical Cluster Analysis (HCA)

In this study, HCA was carried out using the abundances of bioactive compounds (carotenoids, polyphenols, and ascorbic acid) of different rosehip flesh (Figure 4a) and seed (Figure 4b) species.



(**a**) (**b**)

Figure 4. Hierarchical cluster analysis (HCA) of different organic rosehip flesh (**a**) and seed (**b**) samples based on 19 variables. Investigated samples are clustered using Euclidean distance and Ward's method. Abbreviations: RV-F: *Rosa villosa*-flesh; RRR-F: *Rosa rugosa* 'Rubra'-flesh; RRA-F: *Rosa rugosa* 'Alba'-flesh; RC-F: *Rosa canina*-flesh; RR-F: *Rosa rugosa*-flesh; RV-S: *Rosa villosa*-seeds; RRR-S: *Rosa rugosa* 'Rubra'-seeds; RRA-S: *Rosa rugosa* 'Alba'-seeds; RC-S: *Rosa canina*-seeds; RR-S: *Rosa rugosa*-seeds.

Based on the HCA, flesh samples were grouped into three clusters (C1, C2, and C3) (Figure 4a). The first cluster (C1) was formed by RRA-F (*Rosa rugosa* 'Alba'-flesh), RC-F (*Rosa canina-flesh*), and RR-F (*Rosa rugosa*-flesh) (flesh samples with highest contents of *p*-coumaric acid, ferulic acid, luteolin, and quercetin); the second cluster (C2) by RRR-F (*Rosa rugosa* 'Rubra'-flesh) (sample with high contents of chlorogenic acid, caffeic acid, and quercetin-3-*O*-glucoside). The RV-F (*Rosa villosa*-flesh) was grouped separately as the third cluster (C3) (sample with highest contents of carotenoids (sum), individual carotenoids, flavonoids (sum), and rutin).

As shown in Figure 4b, the seed samples were also grouped into three clusters (C1, C2, and C3). The first cluster (C1) was formed by RR-S (*Rosa rugosa*-seeds) and RRA-S (*Rosa rugosa* 'Alba'-seeds) (seed samples with highest contents of ascorbic, gallic, and ferulic acids); the second (C2) cluster by RC-S (*Rosa canina*-seeds) (sample with highest contents of polyphenols (sum), chlorogenic, and caffeic acids, flavonoids (sum) and individual flavonoids such as rutin, kaempferol-3-*O*-glucoside and quercetin) and the third cluster (C3) by RRR-S (*Rosa rugosa* 'Rubra'-seeds) and RV-S (*Rosa villosa*-seeds) (seed samples with high contents of carotenoids (sum) and individual carotenoids, such as α-carotene and lutein).

HCA results suggest that RV flesh and RC seeds can be considered as promising raw material for future researches and food and/or pharmaceutical industries.

3.7. Correlation Analysis

In this study, a correlation analysis was carried out between the total polyphenols content, total flavonoids content, total phenolic acids content, total carotenoids content, and ascorbic acid content of organic rosehips flesh and seeds (Table 4). The results of correlations indicated a very strong positive relationship between total polyphenols content and total phenolic acids content in rosehip flesh and seeds (r = 0.96 and r = 0.98, respectively). Negative correlations were observed between total polyphenols content, total carotenoids content, and ascorbic acid content. In addition, total carotenoids content significantly negatively correlated with total phenolic acids content in the flesh and seed samples (r= −0.86 and r = −0.91, respectively).

Table 4. Correlations between biologically active compounds content in different organic rosehip flesh and seeds.

Compound	TPC	TFC	TPAC	TCC	ACC
	Rosehip flesh				
TPC	1	0.24	0.96 *	−0.79 *	−0.45 *
TFC	0.24	1	−0.50 *	0.55 *	−0.05
TPAC	0.96 *	−0.50 *	1	−0.86 *	−0.39
TCC	−0.79 *	−0.55 *	−0.86 *	1	0.44
ACC	−0.45 *	−0.05	−0.39	0.44	1
Compound	Rosehip seeds				
TPC	1	0.46 *	0.98 *	−0.87 *	−0.28
TFC	0.46 *	1	0.29	−0.10	−0.70 *
TPAC	0.98 *	0.29	1	−0.91 *	−0.16
TCC	−0.87 *	−0.10	−0.91 *	1	0.11
ACC	−0.28	−0.70 *	−0.16	0.11	1

* $p < 0.05$. TPC: total polyphenols content; TFC: total flavonoids content; TPAC: total phenolic acids content; TCC: total carotenoids content; ACC: ascorbic acid content.

4. Conclusions

The findings reveal that the carotenoids in all organically grown rosehip flesh present significantly higher concentrations than in seeds. Furthermore, β-carotene was the predominant carotenoid component in rosehip flesh, and the significantly highest concentration was observed in RV species.

Among the phenolic acids, *p*-coumaric and gallic acids dominated. Overall, the results of this study demonstrate that the highest content of total phenolic occurred in *Rosa canina* fruit species. According to the flavonoids' composition, *Rosa villosa* flesh and *Rosa canina* seeds were found to be the most valuable, and they accumulated the significantly highest contents of these compounds. The content of ascorbic acids in *Rosa rugosa* was highest both in flesh and seeds.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/15/5337/s1, Figure S1: Standard curves for all carotenoids identified in experiment in organic rose fruits and seeds experiment, Figure S2: Standard curves for all phenolic acids identified in experiment in organic rose fruits and seeds experiment, Figure S3: Standard curves for all flavonoids identified in experiment in organic rose fruits and seeds experiment.

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