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COMPARATIVE ANALYSIS OF WILD AND CULTIVATED ROSEHIP FROM BULGARIA

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Abstract

The aim of this study was to compare the composition of wild *Rosa canina* (Kiustendil region, 2022) and cultivated rosehips Plovdiv-1 (Gotse Delchev region, 2022). The results demonstrated that the cultivated rosehips possessed more fibers and minerals, and were more abundant in polyunsaturated fatty acids. The Plovdiv-1 rosehips were characterized by lower fat content and more pronounced antioxidant properties. The wild type had higher vitamin C content. The morphological characterization indicated that the fruits of cultivated rosehips were longer, with less seeds and hairs. The results of the antimicrobial activity tests demonstrated that both methanol extracts possessed significant inhibitory activity against *Micrococcus luteus* 2YC-YT, moderate inhibitory effect on *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* NBIMCC 8632, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Pseudomonas aeruginosa* ATCC 9027, and low inhibitory activity against *Bacillus amyloliquefaciens* 4BCL-YT, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 19433, *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* NBIMCC 1672 and *Klebsiella pneumoniae* ATCC 13883. The extracts did not show antifungal activity, except against *Rhizopus* sp. and *Fusarium moniliforme* ATCC 38932, in which the inhibitory activity was low. The obtained results showed that cultivated rosehips are promising for supplementing various types of food products.

Keywords: wild rosehip, cultivated rosehip, antimicrobial activity, bioactive compounds, antioxidant activity

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Introduction

A large amount of biologically active compounds are found in a significant proportion of medicinal herbs and berries. According to Fuentes *et al.* (2019), in the food, medicinal and cosmetic industries in many places around the world, the fruits of the rosehip (*Rosa canina*) are commonly used and have proven beneficial properties. In Appendix No. 1 to Law on Medicinal Plants in 2000, 26 species of wild rosehip were described as naturally occurring in Bulgaria, and, the following are included: Bush rosehip (*Rosa corymbifera*), Gallic rosehip (*Rosa gallica*) and Common rosehip (*Rosa canina*). Different types of rosehips are hard to be distinguished from each other because their fruits are similar.

The wild rosehip (*Rosa canina*) is a perennial shrub, resistant to harsh climatic conditions, growing on rocky and sloping sites, on poor soils and with limited access to water (Demir and Özcan, 2001). Rosehip (*Rosa canina*) fruits are 14.0-28.8 mm long, 13-20 mm in diameter and weigh 1.2 to 2.7 g. The percentage of the pulp to the total weight of the fruit varies from 42.9% to 66.5%, which is due to the differences in the species (Soare *et al.*, 2015; Igual *et al.*, 2022). Carotenoids, phenolic compounds and polysaccharides in the fruits of rosehip *Rosa canina* are natural antioxidants accepted as functional compounds with good properties for human health. According to some researchers, the content of vitamin C in rosehip fruits ranges from 122 to 1230 mg/100 g (Soare *et al.*, 2015). Oprica *et al.* (2015) suggested that the content of ascorbic acid in the fruits of *Rosa canina* varies with the degree of maturity of the fruits.

The mineral composition of the fruits of *Rosa canina* includes a large number of essential mineral elements, such as phosphorus, potassium, calcium, magnesium, iron, zinc, copper and manganese. The organic acids profile of the fruits includes malic, quinic, tartaric and citric acids (Cunja *et al.*, 2016).

According to some researchers, the fruits have a total phenolic content ranging between 290 and 1385 mg/100 g (Koczka *et al.*, 2018). Carotenoids, such as lycopene, β-cryptoxanthin, β-carotene, rubixanthin, gazaniaxanthin, and zeaxanthin, are also identified in the rosehip berries (Patel, 2013). Therefore a high antioxidant activity in fruits, due to the presence of flavonoids, tannins, terpenoids, xanthonoids and glycerol glycoside, is expected with considerable variability between species.

From the wild rose (*Rosa canina*), the variety "Plovdiv-1" was cultivated and patented by Prof. M. Popova from the Agricultural University in Plovdiv in the 1970. The bush is tall, the shoots are arched and have no thorns. The variety has large fruits, shiny, oblong, and raspberry-red, with a weight of one fruit from 1.70 to 2.40 g. It has high fertility; often 10-12 to 20 fruits are formed from one inflorescence. The fruits of the cultivated rosehip are large, dark red, with a large fleshy part (from 60 to 62.5%) and excellent taste. The fruits are distinguished by a relatively high content of vitamin C - from 100 to 2000 mg/100 g (Popova, 2009). The fruits of the rosehip variety "Plovdiv-1" are of the species *Rosa canina*, family *Rosaceae*. They are rich in the fleshy part and are characterized by a relatively high content of vitamin C and excellent taste. The fruits of the "Plovdiv-1" rosehip variety are commonly used in the production of jams and tea, and in the production of husk (dried fleshy part).

A number of studies were conducted to compare different types of rosehips, which show similar quality profiles but significant differences ($p<0.05$) in the content of bioactive compounds in the fruit (Celik *et al.*, 2009; Demir and Özcan, 2001; Fromm *et al.*, 2012; Cunja *et al.*, 2016; Güney, 2020; Igual *et al.*, 2022). This gives us reason to assume that there will be a difference in the content and concentrations of bioactive compounds in the fruits of wild (*Rosa canina*) and cultivated "Plovdiv-1" rosehips. They will be influenced by the type and geographic area of collection.

The aim of the present study is to determine the differences in the concentrations of bioactive compounds, antioxidant and antimicrobial activity of the two types of rosehips - wild (*Rosa canina*) and cultivated "Plovdiv-1".

Materials and methods

Materials

In the present study, two types of dry rosehip fruits were used - wild rosehip (*Rosa canina*) from the region of Kiustendil harvested in 2022 and purchased from the commercial network, and cultivated rosehip variety "Plovdiv-1" from the region of Gotse Delchev (Blagoevgrad, Bulgaria), harvested in 2022 and purchased from a private producer. The chosen regions are from the same south-west part of Bulgaria and are at similar altitude rates.

The fruits were stored in double paper bags in a wooden cabinet for one year at room temperature, away from direct sunlight or other heat sources. The glyceride oil was obtained by cold pressing of rosehip seeds (by means of a screw press at a pressure of 70-75 atm, at a working chamber temperature of 50-55°C). The rest of the rosehip fruits was processed to flours. The fruits of the rosehips were ground on a laboratory mill (Model PRO 02; 2600 rpm) to 1-2 mm, after previously separating the seeds from the fleshy part (husk).

Morphological description of rosehips

The main morphological rosehip characteristics (shape, length, width, shape index, weight, and percentage of seeds, flakes and hairs) were determined for 100 fresh pseudofruits for each of the two studied species. Using a digital caliper (HBM 6212, 0-150 mm), the length of the hips (L) from the stalk to the calyx was measured. Hip width (D) was determined at the widest part of the pseudofruit, perpendicular to its length. The shape index was calculated as the ratio of length to width (L/W). Fruit weight was measured by Kern weighing balance (Switzerland), model LE011.

Fatty acid profile of rosehip seed oils

For fatty acid analysis, oils were transmethylated by an alkaline method. Roughly 10-20 mg oil (1-2 drops from a Pasteur pipette) were dissolved in 2.5 mL of heptane. Then, 2.5 mL of a 2N KOH in methanol were added and stirred for one minute. After phase separation, the upper phase was directly injected into a gas-chromatograph at a split rate of 1/100. Separation was performed on a Thermo Trace GC using a Supelco SP2560 column (100 m long, 0.25 mm ID). The temperature program was: 100°C for 1 min, and 5°C/min to 240°C, 5 min hold. The injector and detector (FID) temperatures were set at 250°C. Quantification was done by area normalization of

uncorrected peak areas. Peaks were identified by comparison with Supelco 37 component standard.

Phytosterol content of rosehip oils and flours

100 mg of extracted oil was saponified according to Ulberth and Reich (1992). Separation was performed by GC using a Thermo FOCUS GC with FID detection and a SLB-5 column (Supelco, 30 m long, 0.25 mm ID) and a Split injection at a rate of 1/25. Temperature program was 100°C for 1 min then 30°C/min to 260°C and 3°C/min to 300°C – 3 min hold. Alpha-cholestane was used as an internal standard and all phytosterols were quantified against a calibration function of cholesterol. Phytosterols were previously identified by GC-MS.

Tocopherol content of rosehip flours

Tocopherols were measured from extracted oil according to DGF method F-II 4a (00) (Deutsche_Gesellschaft_für_Fettwissenschaft, e.V., Standardmethode F-II 4a (00), in Deutsche Einheitsmethoden 2000, Wissenschaftliche Verlagsgesellschaft mbH: Stuttgart). In brief, 1 g of oil was weighed to the nearest 0.1mg into a calibrated flast and filled to 25 mL with hexane. 20 µL of this solution was injected into a HPLC consisting of a Supelco NH2 column (25 cm x 4.6 mm) and a fluorescence detector (EX 295 nm, EM 340 nm). Mobile phase was 70% hexane, 30% ethylacetate. Calibration was performed by external calibration according to the DGF method.

Lycopene and β-carotene content of rosehip flours

Rosehip flour (1 g) was mixed with 50 mL acetone for 20 min in the dark, and the mixture was filtered. Carotenoids from the remaining material were subsequently extracted in the same way twice by mixing with 30 ml acetone for 20 min and combining the filtrates in a separating funnel. Petroleum ether (75 ml) was added, and the organic phase was washed three times with 50 ml water. Remaining water was removed with anhydrous sodium sulphate, and the volume was made up to 100 ml with petroleum ether.

The concentrations of lycopene and β-carotene were determined spectrophotometrically (Perkin Elmer – Lambda 25) using equations according to (Lime *et al.*, 1957):

$$\beta\text{-carotene } (\mu g/mL) = 4.624 \times A_{450} - 3.091 \times A_{503} \quad (1)$$

$$\text{lycopene } (\mu g/mL) = 3.956 \times A_{450} - 0.806 \times A_{503} \quad (2)$$

Protein content of rosehip flours

The soluble protein content was determined using the colorimetric method proposed by Lowry *et al.* (1951). It is based on the colorimetric measurement of the blue color that results from: 1) the reaction of the protein with copper tartrate ions in an alcoholic medium and 2) the reduction of phosphomolybdic/phosphotungstic acid (Folin phenol reagent) acid by the available tyrosine and tryptophan. The absorbance

is read with a spectrophotometer (HALO RB-10 Spectrophotometer) at a wavelength of λ 750 nm relative to the control (blank) sample. The amount of bound protein is calculated according to a previously prepared standard curve with bovine serum albumin (BSA).

Moisture of rosehip flours

It was done by drying at a temperature of 105°C to a constant weight.

Fat content of rosehip flours

It was analysed by extracting the sample with an organic solvent (petroleum ether) for 12 hours in a Soxhlet extractor (BNS ISO 6492:2007). After evaporation of the solvent, the flask with the oil is dried in a dryer at 100-105 ºC to a constant mass. The oil content of the pure sample in % relative to the absolute dry matter is calculated by the formula 3.

$$X = \frac{M \cdot 100}{(100 - B)} \qquad (3)$$

where: M - oiliness of the clean material sample at the available moisture, %; B – moisture content, %.

Pectin content of rosehip flours

It was calculated by sequentially treating the ground fruit with petroleum ether, hexane and 95% ethanol in a Soxhlet apparatus to remove dyes, fats and waxes. The AUA content and the degree of esterification (DE) of pectic polysaccharides obtained from rosehip fruits was determined by the titrimetric method, according to the Food Chemical Codex (2004), slightly modified for using Hinton's indicator.

Tannins content of rosehip flours

Tannins content was determined by exhaustive extraction with hot water under reflux and titration of the resulting extract with 0.1 N $KMnO_4$ using indigo carmine as indicator according to the State Pharmacopoeia of the USSR, XI, 1990.

Total phenolic content of rosehip flours

Rosehip fruits were extracted with 70 % (v/v) ethanol using ultrasound-assisted extraction for 20 min at 40 °C at 40 kHz. The solid to liquid ratio was 1:20 w/v. The extraction procedure was repeated twice. The obtained extracts were filtered through filter paper. The content of total polyphenols was determined as 1 mL Folin Ciocalteu reagent diluted five times was mixed with 0.2 mL sample and 0.8 mL 7.5% Na_2CO_3. The reaction was performed for 20 min at room temperature in darkness. Then the absorbance was measured at 765 nm against blank. The results were expressed as mg equivalent of gallic acid (GAE) per g dry weight (dw), according to calibration curve, build in range of 0.02 - 0.10 mg gallic acid (Ivanov *et al.*, 2014).

Total ascorbic content of rosehip flours

Official Method for the Analysis of Vitamin C in Juices by the 2,6-Dichloroindophenol Titrimetric Method (AOAC Method 967.21) was applied.

Total fibre content

The total, insoluble and soluble dietary fibres was determined using K-TDFR-100A enzyme kit (Megazyme, Ireland), according to AOAC method 991.43 "Total, soluble and insoluble dietary fibres in foods" (1991) and AACC method 32-07.01 "Determination of soluble, insoluble and total dietary fibres in foods and food products" (1991).

Glucose and reducing sugars content

Glucose content was established by HPLC analysis and reducing sugars were evaluated by the PAHBAH method both described by Petkova and Ognyanov (2018).

Minerals

The content of micro and macro minerals was determined after mineralization of the samples at a temperature of 450°C. The residue was dissolved in concentrated HCl (37%) and the resulting solution was immediately evaporated. The dry residue was dissolved in 0.1 mol/L HNO_3 and transferred to a Perkin Elmer/HGA 500 atomic absorption spectrometer (AAS) (Norwalk, USA) for elemental content determination. The flame atomic absorption spectroscopy (FAAS) parameters were: Na – 589.6 nm; K – 766.5 nm; Mg – 285.2 nm; Ca – 317.0 nm; Zn – 213.9 nm; Cu – 324.7 nm; Fe – 238.3 nm; Mn – 257.6 nm. Identification is by standard solutions of salts of the respective metals. The concentration was calculated from a calibration curve constructed using a standard saline solution with a concentration of 1 µg/mL.

Antioxidant activity of rosehip flours by α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging assay

The radical scavenging capacity was determined according to the method of Brand-Williams *et al*. (1995) with the following modification: 2250 µL of DPPH solution (2.4 mg of DPPH in 100 mL of methanol) and 250 µL of extract (base extract or fraction) were successively dosed into the cuvette. A blank was prepared similarly using methanol instead of extract. After incubation for 15 min at room temperature in darkness, the absorbance of the reaction mixture was measured at 515 nm. The results obtained were presented as Trolox equivalents µmol TE/100 g.

Antioxidant activity of rosehip flours by ferric reducing ability of plasma (FRAP) assay

The reagent was prepared by mixing previously prepared 0.3 M acetate buffer pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3 \times 6H_2O$ in a ratio of 10:1:1. The test extract (0.1 mL) was added to 3 mL of FRAP reagent. The reaction mixture was incubated for 5 min at 37 °C in the dark. The absorbance of the coloured substance formed was measured at a wavelength of 593 nm in compliance with Benzie and Strain (1996).

Antimicrobial activity of rosehip flours - test microorganisms

Twenty microorganisms, including six Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus amyloliquefaciens* 4BCL-YT, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* NBIMCC 8632, *Enterococcus faecalis* ATCC

19433, *Micrococcus luteus* 2YC-YT), six Gram-negative bacteria (*Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* NBIMCC 1672, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Pseudomonas aeruginosa* ATCC 9027), two yeasts (*Candida albicans* NBIMCC 74, *Saccharomyces cerevisiae* ATCC 9763) and six fungi (*Aspergillus niger* ATCC 1015, *Aspergillus flavus*, *Penicillium chrysogenum*, *Rhizopus* sp., *Mucor* sp. – plant isolates, *Fusarium moniliforme* ATCC 38932) from the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria, were selected for the antimicrobial activity test.

Antimicrobial activity of rosehip flours - Culture media

Luria-Bertani agar medium supplemented with glucose (LBG agar) was used for cultivation of test bacteria. A quantity of 50 g of LBG-solid substance mixture (containing 10 g tryptone, 5 g yeast extract, 10 g NaCl, 10 g glucose and 15 g agar) was dissolved in 1 L of deionized water, pH 7.5±0.2. Malt extract agar (MEA). MEA was used for cultivation of test yeasts and fungi. A quantity of 50 g of the MEA-solid substance mixture (containing 30 g malt extract, 5 g mycological peptone and 15 g agar) was dissolved in 1 L of deionized water, pH 5.4 ± 0.2. The culture media were prepared according to the manufacturer's instructions (Scharlab SL, Spain) and autoclaved at 121°C for 20 min before use.

Antimicrobial activity of rosehip flours – assay

Methanol extract of the rosehip flour was prepared as follows: 1 g rosehip flour was poured in 10 mL methanol for 48 h. Then it was filtered and the antimicrobial activity of rosehips methanol extracts was determined by the agar well diffusion method according to Tumbarski *et al.* (2018). The bacteria *B. subtilis*, *B. amyloliquefaciens* and *M. luteus* were cultured on LBG agar at 30°C for 24 h, while *S. aureus*, *L. monocytogenes*, *L. innocua*, *E. faecalis*, *E. faecium*, *S. enteritidis*, *Klebsiella* sp., *E. coli*, *P. vulgaris* and *P. aeruginosa* were cultured on LBG agar at 37°C for 24 h. The yeast *C. albicans* was cultured on MEA at 37°C, while *S. cerevisiae* was cultured on MEA at 30°C for 24 h. The test fungi *A. niger*, *A. flavus*, *Penicillium sp.*, *Rhizopus sp.*, and *F. moniliforme* were grown on MEA at 30°C for 7 days or until sporulation. The bacterial/yeast inocula were prepared by homogenization of a small amount of biomass in 5 mL of sterile 0.5% NaCl. The fungal inocula were prepared by the addition of 5 mL of sterile 0.5% NaCl directly into the cultivation tubes. After stirring by vortex V-1 plus (Biosan, Latvia), the fungal inocula were filtered and replaced in another tubes before use. The number of viable cells and fungal spores was determined using a bacterial counting chamber Thoma (Poly-Optik, Germany). Their final concentrations were adjusted to 108 cfu/mL for bacterial/yeast cells and 105 cfu/mL for fungal spores, and then inoculated in preliminarily melted and tempered at 45-48°C LBG/MEA. Next, a volume of 18 mL of inoculated media were transferred in sterile Petri plates (d = 90 mm) (Gosselin™, France) and allowed to harden. Then six wells (d = 6 mm) per plate were cut and triplicates of 60 µL of the methanol extracts were pipetted into the agar wells. The Petri plates were incubated at identical conditions. The antimicrobial activity was determined by measuring the diameter of the inhibition zones (IZ) around the wells on the 24^{th} and 48^{th} hour of

incubation. Test microorganisms with IZ of 18 mm or more were considered sensitive; moderately sensitive were those in which the IZ were from 12 to 18 mm; resistant were those in which the IZ were up to 12 mm or completely missing.

Statistical analysis

Data from triplicate experiments were processed with MS Office Excel 2010 software using statistical functions to determine the standard deviation (± SD). One-way ANOVA was applied in order to determine differences between samples and maximum estimation error at significance levels $p<0.05$.

Results and discussion

Morphological description of "Plovdiv-1" rosehip and wild (Rosa canina) rosehips

The obtained information on the main morphological characteristics, including the location of the spikes, are given in Table 1.

Table 1. Morphological description of "Plovdiv-1" rosehip and wild (*Rosa canina*) rosehips.

	Cultivated rosehip "Plovdiv-1"	**Wild rosehip (*Rosa canina*)**
Location	Gotse Delchev	Kiustendil
Altitude rank, m	580	540
Shape	elliptical	round
Length (L), mm	25.80±0.13	15.89±0.14
Width (D), mm	11.6±0.12	10.34±0.14
Shape index, L/D	2.20±0.10	1.56±0.18
Weight, g	1.60±0.14	0.82±0.13
Flakes, %	39.40±0.18	35.50±0.19
Seeds, %	56.80±0.25	58.40±0.21
Hairs, %	3.80±0.21	6.10±0.19

The fruits of the cultivated rosehip variety "Plovdiv-1" were large, dark red, with a large fleshy part, unlike the wild rosehips. It was found that the shape of the wild rosehip (*Rosa canina*) was round, and that of the cultivated variety "Plovdiv-1" was elliptical. As for the fruit shape, rosehips weight was quite variable and ranged from 0.82 g to 1.60 g, showing almost twice the values in the cultivated rosehip. The weight of the wild rosehip (0.82 g) was lower than reported by other authors (Igual *et al.*, 2022), which can be explained by the specifics of the harvesting area and growing conditions. The width of the two studied types of rosehips was close in value, in contrast to the length, where there were significant differences ($p<0.05$). In cultivated rosehip "Plovdiv-1", significantly longer pseudofruits (25.80 mm) were observed compared to those of wild *Rosa canina* (15.89 mm). The shape index of the cultivated cultivar "Plovdiv-1" (2.23, corresponding to longer fruits) was greater than that of the wild rosehip (1.56, indicating a round shape). Similar results were obtained by Celik *et al.* (2009), for the shape and sizes of different types of rosehips.

Contrast values were also observed in the remaining anatomical parts, such as seeds and hairs. The differences in the percentage of seeds and flake content in both species, was significant ($p<0.05$). The content of hairs in the wild rosehip (6.1%) was greater than that of the cultivated variety "Plovdiv-1" (5.8%). The fleshy part of the fruit ranged from 35.5% to 39.4% of the weight of the fruit, and it was higher (39.4%) in the "Plovdiv-1" variety. These differences were probably due not only to the type of rosehips, but also to the different microlocation, climatic and soil conditions of their cultivation.

Chemical composition of cultivated "Plovdiv-1" rosehip and wild (Rosa canina.) rosehip oils

The two extracted oils were examined, and the obtained results are presented in Table 2.

Table 2. Chemical composition of "Plovdiv-1" rosehip and wild (*Rosa canina*) rosehip oils.

Fatty acid profile, %	**Cultivated rosehip "Plovdiv-1"**	**Wild rosehip (*Rosa canina*)**
C16:0	4.9±0.5	6.6±0.5
C16:1	0.1±0.0	0.1±0.0
C18:0	3.2±0.6	4.8±0.5
C18:1n9	14.6±1.1	23.6±1.3
C18:1n7	0.4±0.1	0.7±0.1
C18:2n6c	50.8±4.5	40.4±3.7
C20:0	1.1±0.1	0.2±0.0
C20:1	0.3±0.1	0.1±0.0
C18:3n3	23.7±0.2	22.4±0.5
C22:0	0.1±0.0	0.4±0.1
C24:0	0.1±0.0	0.1±0.0
Sterol content, mg/100 g		
Campesterol	tr*	tr*
Stigmasterol	nd*	tr*
β-Sitosterol	183±5	42±4

*tr – traces; nd – not detected

The composition of rosehip seed oils of the studied rosehips corresponds to the results reported in the literature (Turan *et al.*, 2018; Güney, 2020). The obtained results showed a significantly higher ($p<0.05$) amount of unsaturated fatty acids (90.0%) in the "Plovdiv-1" variety compared to the wild-growing variety (87.3%). Our research confirmed the conclusion made by Dąbrowska *et al.* (2019) who showed the correlation between the cold extraction of the oil and its higher content of unsaturated fatty acids. Probably this is due to the preservation of the chemical structure and integrity of these fats during the process, because the cold extraction method (cold pressing) involves mechanical pressing of the seeds or fruit at lower temperatures, usually below 50°C. These temperatures are lower than those used in Soxhlet extraction which can promote oxidation and hydrolysis reactions leading to the formation of free radicals and the breakdown of unsaturated fats. The obtained

values are among the highest reported in Europe and are comparable only to some rosehip oils from Hungary (Dąbrowska *et al.*, 2019) and Turkey (Ilyasoğlu, 2014). These results could be explained with the type of lipid extraction, specific genetic characteristics of the studied varieties, agro technical measures applied in rosehip cultivation (*i.e.* fertilization) and different absorption of certain nutrients from the soil, etc.

A deeper analysis of the obtained results shows that the oil extracted from Plovdiv-1 variety rosehips could be considered as an excellent source of the essential for the human body α-linolenic acid (C18:3n3, omega-3 fatty acid) and linoleic acid (C18:2n6c, omega-6 fatty acid). This determines their high biological value, because omega-3 and omega-6 fatty acids are essential polyunsaturated fats that play a crucial role in the human body. They are called "essential" because the human body cannot synthesize them and must be obtained through food. Omega-3 fatty acids are associated with cardiovascular health. They have been shown to reduce the risk of heart disease by lowering blood pressure, reducing triglycerides and improving total cholesterol levels. Omega-3 and omega-6 fatty acids boost the immune system, participate in the production of immune system components and help modulate the immune response (Dąbrowska *et al.*, 2019). It is confirmed by the presence of important quantity of oleic acid (C18:1n9, omega-9 fatty acid) which is one of the most studied fatty acids by some authors (Güney, 2020). Due to the high level of linoleic and oleic acid, rosehip glyceride oil has a higher oxidative stability than other unsaturated oils and can be used as an additive in the formulation of functional foods, giving a special flavour to food products.

The presence of phytosterols, in both rosehip oils, was determined only by β-sitosterol which content was four times more abundant in the cultivated Plovdiv-1" rosehip oil. These results are different by those obtained by Ilyasoğlu (2014) and Turan *et al.* (2018) which fact can be explained by variations of listed above factors in cultivation and also by the ecological changes which occur worldwide.

Chemical composition of "Plovdiv-1" rosehip and wild (Rosa canina) rosehip flours

A detailed analysis of the composition of the two types of rosehip flour was carried out. The obtained results are presented in Table 3.

The obtained results show certain differences in the content of mineral elements in cultivated "Plovdiv-1" and wild rosehip (*Rosa canina*). The amount of K, Na, Zn, Fe and Cu is higher in cultivated rosehip variety "Plovdiv-1" than in wild rosehip (*Rosa canina).* A higher content of Mg and P was observed in the wild rosehip than in the cultivated rosehip. Similar results for differences in the mineral composition were obtained by Kizil *et al.* (2018) investigating fruits of cultivated and wild rosehips in Turkey. An analysis made by (Medveckienė *et al.*, 2022) indicates that the mineral composition of the rosehip also depends on the stage of maturity of the pseudofruits. A large part of the analysed indicators of the chemical composition of rosehip flour are contained in significant quantities in the cultivated variety Plovdiv-1, and the total polyphenols, β-carotene, lycopene, reducing sugars, tannins, glucose, pectin and crude protein exceed the values of the wild-growing variety by several times.

Wild rosehip (*Rosa canina*) stands out for its vitamin C content (245.6 mg/100g against 153.8 mg /100 g for the cultivated type).

Table 3. Chemical composition of "Plovdiv-1" rosehip and wild (*Rosa canina*) rosehip flours.

	Cultivated rosehip "Plovdiv-1"	**Wild rosehip (*Rosa canina*)**
Total dietary fibers, %	38.75 ± 1.47	35.49 ± 1.63
Insoluble dietary fibers, %	22.56 ± 1.38	21.08 ± 1.44
Soluble dietary fibers, %	17.82 ± 1.59	15.26 ± 1.68
Pectin, %	21.2± 1.1	13.4 ± 1.0
Glucose, g/100g	4.59 ± 0.33	2.58 ± 0.13
Reducing sugars, g/100g	13.51 ± 1.13	5.49 ± 0.77
Campesterol, mg/100 g	14 ± 1	traces
Stigmasterol, mg/100 g	9 ± 1	Not detected
β-Sitosterol, mg/100 g	57 ± 3	33 ± 5
Moisture, %	11.27 ± 1.13	13.45 ± 1.01
Fat, %	1.50 ± 0.71	2.88 ± 0.31
Protein, %	7.38 ± 1.00	1.60 ± 0.09
Vitamin C, mg/100g	153.8 ± 13.1	245.6 ± 13.3
Lycopene, μg/g	220.28 ± 15.15	44.28 ± 1.15
β-carotene, μg/g	178.27 ± 15.14	28.70 ± 1.10
α-tocopherol (mg/100 g)	7.61 ± 0.30	8.32 ± 0.13
γ-tocopherol (mg/100 g)	4.20 ± 0.14	5.73 ± 0.12
Total polyphenols mg GAE/g	8400 ± 235	3120 ± 211
Tannins, %	13.24 ± 1.14	5.45 ± 1.01
P, %	0.38 ± 0.01	0.41 ± 0.02
K, mg/kg	8460.66 ± 11.17	1039.56 ± 10.10
Na, mg/kg	216.74 ± 12.41	93.74 ± 8.10
Mg, mg/kg	2 148.98 ± 21.16	2331,7 ± 33.14
Zn, mg/kg	12.91 ± 1.10	5.56 ± 0.91
Fe, mg/kg	89.37 ± 1.17	26.20 ± 1.10
Cu, mg/kg	6.27 ± 1.1	4.35 ± 0.91
Pb, mg/kg	0.0704 ± 0.0001	0.052 ± 0.0001
Ni, mg/kg	0.314 ± 0.016	1.060 ± 0.010

Rosa canina rosehip fruits are rich in vitamin C. The presence of organic acids and flavonoids in rosehip fruits prevent oxidation of the vitamin, making them a very suitable source of vitamin C. Nojavan *et al.* (2008) proved that the content of vitamin C in dried fruits of *Rosa canina* was higher (211 mg/100 g) than that of fresh fruits (31.48 mg/100 g). Some authors suggest that the level of vitamin C in rosehips may depend on soil conditions and climate as well as on their maturity (Nojavan *et al.*, 2008; Oprica *et al*., 2015). The fruits of rosehips of both species (cultivated "Plovdiv-1" and wild *Rosa canina*) contain high amounts of dietary fibers, and in the cultivated one they are slightly higher (38.75±1.47%) than the wild rosehips (35.49±1.63%) (Table 3).

In both types of rosehips, the proportion of insoluble fibres was higher. The presence of fiber in the studied fruits is similar to the data found in the literature (Igual *et al.*, 2022). The origin of the raw material and climatic features is a possible reason for the differences in the content of phytosterols compared to the data of other authors (Ilyasoğlu, 2014; Turan *et al.*, 2018). Regarding tocopherol content, the values are relatively similar to those of Fromm *et al.* (2012).

Antioxidant activity of "Plovdiv-1" rosehip and wild (Rosa canina) rosehip flours

Antioxidant activity of the rosehip flours was determined by two different methods, and the obtained results are presented in Figure 1.

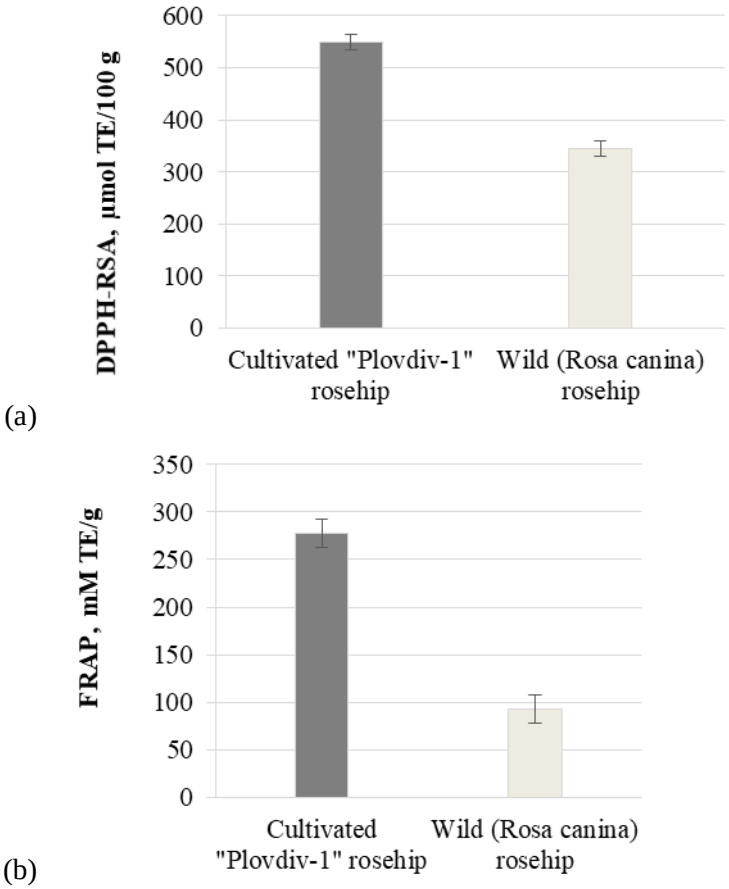

Figure 1. Antioxidant activity of "Plovdiv-1" rosehip and wild (*Rosa canina*) rosehip flours, determined by (a) DPPH method, and (b) FRAP method.

Antioxidants play an important role in human health by protecting cells from damage caused by free radicals. Antioxidants reduce oxidative damage to blood vessels and lipids, improve the activity of the cardiovascular system, lower blood pressure, and reduce cholesterol levels and the risk of atherosclerosis (Güney, 2020). The

antioxidant activity of the fruits of cultivated variety "Plovdiv-1" and wild rosehips was determined. It was established that with the two analysed methods, a higher antioxidant activity was observed in the cultivated rosehip variety "Plovdiv-1". The difference in antioxidant activity between the two types of rosehips was significant by both methods of determination. In cultivated rosehip "Plovdiv 1" according to DPPH method the antioxidant activity was 548.99 µmol TE/100 g, and according to FRAP method – 277.75 mM TE/g dry sample, while in wild rosehip the values were respectively: 345.27 µmol TE/100 g (DPPH) and 92.59 mM TE/g dry mass sample g (FRAP). This is probably due to the higher amount of substances with antioxidant potential presented in Table 3. These results are in accordance with previous studies conducted on *Rosa canina* L. fruits, which showed that they have strong antioxidant activity. For example, Shameh *et al.* (2019) investigated different *Rosa canina* fruits from subspecies and found significant differences in antioxidant activity between genotypes belonging to different *Rosa taxa*. They indicated that among the studied species, *Rosa hemisphaeri* had the lowest antioxidant activity and *Rosa canina* had the highest antioxidant activity. Cunja *et al.* (2016) investigated the biological activity of the fruits of the genus Rosa and found that the fruits of *Rosa canina* have the highest antioxidant activity among other species. Along with the species and genotype, the biological activity of the fruits is influenced by the conditions of cultivation, the degree of ripening, the time of harvesting and a number of other factors. These results show higher values compared to those obtained for *Fumaria* species (Ivanov *et al.*, 2014) and Cornelian cherries (Petkova and Ognyanov, 2018).

Antimicrobial activity of "Plovdiv-1" rosehip and wild (Rosa canina) rosehip flours methanol extracts

One of the potential biological activities demonstrated by the bioactive compounds is their ability to exhibit antimicrobial activities. Our research revealed interesting results in this direction, presented in Table 4.

The highest inhibitory activity of both rosehip extracts was observed against the gram-positive microorganism *Micrococcus luteus* 2YC-YT. Against *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* NBIMCC 8632, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380 and *Pseudomonas aeruginosa* ATCC 9027 have a moderate inhibitory effect, and on *Bacillus amyloliquefaciens* 4BCL-YT, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* NBIMCC 1672 and *Klebsiella pneumoniae* ATCC 13883 antimicrobial activity is low. A low antifungal activity was observed in *Rhizopus* sp. and *Fusarium moniliforme* ATCC 38932. Close to the indicated results are those of (Ghendov-Moşanu *et al.*, 2018) for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*.

Table 4. Antimicrobial activity of "Plovdiv-1" rosehip and wild (*Rosa canina*) rosehip flours.

	Cultivated rosehip "Plovdiv-1"	**Wild rosehip (*Rosa canina*)**	**MeOH (control)**
Test microorganisms	**Inhibition zones, mm**		
Bacillus subtilis ATCC 6633	13	13	-
Bacillus amyloliquefaciens 4BCL-YT	10	10	-
Staphylococcus aureus ATCC 25923	10	10	-
Listeria monocytogenes NBIMCC 8632	12	12	-
Enterococcus faecalis ATCC 29212	10	-	-
Micrococcus luteus 2YC-YT	20	21	-
Salmonella enteritidis ATCC 13076	10	11	-
Salmonella typhimurium NBIMCC 1672	10	9	-
Klebsiella pneumoniae ATCC 13883	9	9	-
Escherichia coli ATCC 25922	12	12	-
Proteus vulgaris ATCC 6380	12	13	-
Pseudomonas aeruginosa ATCC 9027	12	12	-
Candida albicans NBIMCC 74	-	-	-
Saccharomyces cerevisiae ATCC 9763	-	-	-
Aspergillus niger ATCC 1015	-	-	-
Aspergillus flavus	-	-	-
Penicillium chrysogenum	-	-	-
Rhizopus sp.	8	8	-
Fusarium moniliforme ATCC 38932	8	8	-
Mucor sp.	-	-	-

Conclusions

The results of the present study showed differences in the composition of the two types of rosehips grown in Bulgaria. Fruits are defined by distinctive morphological characteristics. The results of the present study show that the rosehips of both *Rosa canina* species can be present as a valuable natural source of vitamin C and can be used in products poor in this vitamin. The fruits of the cultivated rosehip variety "Plovdiv-1" are a good source of mineral elements like phosphorus, potassium, sodium, magnesium, zinc and iron. They are also rich in various biologically active substances, such as carotenoids, tannins, phytosterols, polyphenols, and tocopherols. The oil of the cultivated rosehip variety "Plovdiv-1" is rich in unsaturated fatty acids (oleic, linoleic and linolenic) and some basic mineral elements. This suggests that

these fruits can be effectively used in the food industry. The addition of plant materials to various food products allows the latter to be functionalized, according to the legislation of the European Union. The cultivated rosehips fruits are a good source of dietary fibres and polyphenols, and could be successfully applied in different food formulation, such as to enrich the polyphenolic content and to fortify the final product in vitamins and minerals. Taking into account that many food products, such the milk and dairy products, are not rich in biologically active compounds like ascorbic acid, phenolic compounds etc., we consider testing the possibility of using the two *Rosa canina* varieties for their supplementation. The choice of such an approach, which is a first step in developing functional dairy products with potential beneficial effect on human health, is important for society and might have a clear impact on it.

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References

AACC 32-07.01 "Determination of Soluble, Insoluble, and Total Dietary Fiber in Foods and Food Products", Last approved 10-16-91.

AOAC Official Method 967.21 Ascorbic Acid in Vitamin Preparations and Juices 2,6-Dichloroindophenol Titrimetric Method First Action 1967, Final Action 1968.

AOAC Official Method 991.43 “General. Soluble and insoluble dietary fiber in foods', First Action 1991.

Benzie, F., Strain, J. 1996. Ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical biochemistry*, **239**, 70-76.

Brand-Williams, W., Cuvelier, M., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, **28**(1), 25-30.

Celik, F., Kazankaya, A., Ercisli, S. 2009. Fruit characteristics of some selected promising rose hip (*Rosa* spp.) genotypes from Van region of Turkey. *African Journal of Agricultural Research*, **4**(3), 236-240.

Cunja, V., Mikulic-Petkovsek, M., Weber, N., Jakopic, J., Zupan, A., Veberic, R., Stampar, F., Schmitzer, V. 2016. Fresh from the ornamental garden: Hips of selected rose cultivars rich in phytonutrients. *Journal of Food Science*, **81**(2), C369-C379.

Dąbrowska, M., Maciejczyk, E., Kalemba, D. 2019. Rose hip seed oil: Methods of extraction and chemical composition. *European Journal of Lipid Science and Technology*, **121**(8), 1800440.

Demir, F., Özcan, M. 2001. Chemical and technological properties of rose (*Rosa canina* L.) fruits grown wild in Turkey. *Journal of Food Engineering*, **47**(4), 333-336.

Food Chemical Codex - FCC V. (2004). National Academy of Science, Washington DC, 322-323.

Fromm, M., Bayha, S., Kammerer, D., Carle, R. 2012. Identification and quantitation of carotenoids and tocopherols in seed oils recovered from different *rosaceae* species. *Journal of Agricultural and Food Chemistry*, **60**(43), 10733-10742.

Fuentes, L., Figueroa, C.R., Valdenegro, M., Vinet, R. 2019. Patagonian berries: healthy potential and the path to becoming functional foods. *Foods*, **8**(8), 289.

Ghendov-Moşanu, A., Cojocari, D., Balan, G., Sturza, R. 2018. Antimicrobial activity of rose hip and hawthorn powders on pathogenic bacteria. *Journal of Engineering Sciences*, **4**, 100-107.

Güney, M. 2020. Determination of fatty acid profile and antioxidant activity of Rosehip seeds from Turkey. *International Journal of Agriculture Environment and Food Sciences*, **4**(1), 114-118.

Igual, M., García-Herrera, P., Cámara, R.M., Martínez-Monzó, J., García-Segovia, P., Cámara, M. 2022. Bioactive compounds in rosehip (*Rosa canina*) powder with encapsulating agents. *Molecules*, **27**(15), 4737.

Ilyasoğlu, H. 2014. Characterization of rosehip (*Rosa canina* L.) seed and seed oil. *International Journal of Food Properties*, **17**(7), 1591-1598.

Ivanov, I., Vrancheva, R., Marchev, A., Petkova, N., Aneva, I., Denev, P. 2014. Antioxidant ctivities and phenolic compounds in Bulgarian *Fumaria* species. *International Journal of Current Microbiology and Applied Sciences*, **3**(2), 296-306.

Kizil, S., Toncer, O., Sogut, T. 2018. Mineral contents and fatty acid compositions of wild and cultivated rose hip (*Rosa canina* L.). *Fresenius Environ. Bull*, **27**(2), 744-748.

Koczka, N., Stefanovits-Bányai, É., Ombódi, A. 2018. Total polyphenol content and antioxidant capacity of rosehips of some *Rosa* species. *Medicines*, **5**(3), 84.

Lime, B., Griffiths, F., O'Connor, R., Heinzelman, D., McCall, E. 1957. Spectrophotometric methods for determining pigmentation – beta-carotene and lycopene – in ruby red grapefruit. *Journal of Agricultural and Food Chemistry*, **5**(12), 941-944.

Lowry, O., Rosebrough, N., Lewis Farr, A., Randall, R. 1951. Protein measurement with the folin Phenol Reagent. *Journal of Biological Chemistry*, 1**93**(1), 256-275.

Medveckienė, B., Kulaitienė, J., Vaitkevičienė, N., Levickienė, D., Bunevičienė, K. 2022. Effect of harvesting in different ripening stages on the content of the mineral elements of rosehip (*Rosa* spp.) fruit flesh. *Horticulturae*, **8**(6), 467.

Nojavan, S., Khalilian, F., Kiaie, F.M., Rahimi, A., Arabanian, A., Chalavi, S. 2008. Extraction and quantitative determination of ascorbic acid during different maturity stages of *Rosa canina* L. fruit. *Journal of Food Composition and Analysis*, **21**(4), 300-305.

Oprica, L., Bucsa, C., Zamfirache, M. 2015. Ascorbic acid content of rose hip fruit depending on altitude. *Iranian Journal of Public Health*, **44**(1), 138.

Patel, S. 2013. Rose hips as complementary and alternative medicine: overview of the present status and prospects. *Mediterranean Journal of Nutrition and Metabolism*, **6**(2), 89-97.

Petkova, N., Ognyanov, M. 2018. Phytochemical characteristics and in vitro antioxidant activity of fresh, dried and processed fruits of Cornelian cherries (*Cornus mas* L.). *Bulgarian Chemical Communications*, **50**(C), 302-307).

Popova, M. 2009. Rosehip - perspective culture of agriculture. *Center for European Integration and Culture at the Agricultural College*, Plovdiv.

Shameh, S., Alirezalu, A., Hosseini, B., Maleki, R. 2019. Fruit phytochemical composition and color parameters of 21 accessions of five *Rosa* species grown in North West Iran. *Journal of the Science of Food and Agriculture*, **99**(13), 5740-5751.

Soare, R., Bonea, D., Iancu, P., Niculescu, M. 2015. Biochemical and technological properties of *Rosa canina* L. fruits from spontaneous flora of Oltenia, Romania. *Bulletin UASVM Horticulture*, **72**(1), 182-186.

Tumbarski, Y., Deseva, I., Mihaylova, D., Stoyanova, M., Krastev, L., Nikolova, R., Yanakieva, V., Ivanov, I. 2018. Isolation, characterization and amino acid composition

of a bacteriocin produced by *Bacillus methylotrophicus* strain BM47. *Food Technology and Biotechnology*, **56**(4), 546.

Turan, I., Demir, S., Kilinc, K., Yaman, S. O., Misir, S., Kara, H., Genc, B., Mentese, A., Aliyazicioglu, A.,Deger, O. 2018. Cytotoxic effect of *Rosa canina* extract on human colon cancer cells through repression of telomerase expression. *Journal of Pharmaceutical Analysis*, **8**(6), 394-399.

Ulberth, F., Reich, H. 1992. Gas chromatographic determination of cholesterol in processed foods. *Food Chemistry*, **43**, 387-391.