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THE INFLUENCE OF GENOTYPE AND ENVIRONMENTAL FACTORS ON THE NUTRITIONAL COMPOSITION OF *ROSA CANINA* L. FRUITS

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Abstract

This study aimed to determine how factors such as genotype and place of cultivation influence the variation of nutritional compounds that constitute the nutraceutical profile of Rosa canina L. fruits. These fruits can be integrated as a functional food in the daily life of the Romanian population. Twelve genotypes of Rosa canina L. were studied, for which the following average values were determined and recorded: total dry matter (46.54%); titratable acidity (40.32 mg/s fw); reducing sugar content (23.32%); total polyphenols (48.07 mg GAE/100 g fw), ascorbic acid content (7.04 mg/100 g fw); ABTS antioxidant activity (182.48 µMTE/g fw) mg/100 g). The results obtained showed that the determining factor is the genotype that induces significant variations in the content of nutritional compounds. The high values of the nutritional compounds determined in this study justify the high nutritional potential of these fruits originating from spontaneous flora and their use as natural medicines.

Key words: *antioxidant activity, Rosa canina L, total polyphenols, vitamin C.*

INTRODUCTION

For more than 2,000 years, the pseudofruits of *Rosa canina*, known as rose hips, have been used as herbal remedies (Mitroi et al., 2023). Studies on the first uses of *Rosa canina* in traditional medicine date back to ancient Greece. Hippocrates used the fruit in various recipes, but its precise use is unknown. The Roman naturalist Plinius the Elder gave the species the name "dog rose" in the belief that the root of the plant could cure the bite of a rabid dog, and during World War II, Britain, unable to import fresh citrus fruits, used rose hips as an important source of vitamin C (Mabellini et al., 2011).

Rosa canina L. is a shrub belonging to the *Rosaceae* Family, which contains over 100 species widely distributed in Europe, Asia, Africa, the Middle East and North America. This shrub has numerous uses: in food, in natural medicine, ornamental plant, rootstock for the reproduction of the *Rosa* genus or in agri-environmental measures to combat wind deflation (Iancu et al., 2020). Rosa fruits are among the important products in terms of global production and trade as organic foods collected from nature (Pećinar et al., 2021; Soare et al., 2015a).

They vary in size and color, depending on the genotype, and are recognized for their nutritional characteristics (Peña et al., 2023). In recent years, there has been a growing interest in edible wild fruits, including rose hips, which possess several properties that are beneficial to human health (Demir et al., 2021). Fruits rose hips, have nutritional, pharmacological and cosmetic value. They are very rich in vitamin C, containing over 2000 mg of this substance per 100 grams of fruit. Therefore, they are used in the food industry in the production of syrups, teas, jams, and jellies.

Fruits are an excellent source of nutrients and bioactive compounds that are effective in protecting the human body against various diseases related to oxidative stress, such as cancer, Parkinson's disease, Alzheimer's disease and atherosclerosis (Mitroi et al., 2023). Rose hips are highly valued for their taste, color and aroma, in line with their recognition as one of

the richest sources of pro-health compounds (Kayahan et al., 2023). The mixed sweet-sour taste of the fruits is caused by citric acid, ellagic acid and malic acid (Gunaydin & Alibas, 2023). Rosa canina fruits reach physiological maturity in August-September and their nutritional value is given by the high content of vitamin C compared to other fruits (Roman et al., 2013; Demir & Özcan, 2001). In addition to vitamin C, rose hips also contain a series of other phytochemicals such as phenolic compounds, organic acids, pectins, fatty acids, but also vitamins B1, B2, K, PP, E, macro and microelements, etc. (Mitroi et al., 2023).
Numerous studies have reported different levels of total sugars organic acids and bioactive compounds (Akagić et al., 2020; Fetni et al., 2020). Higher concentrations of bioactive compounds and higher levels of antioxidant activity are of great interest and could contribute to the development of functional foods or for preventive or therapeutic use in various pathologies (Peña et al., 2023).
The aim of this work was to identify valuable rosehip genotypes existing in the south-western part of Romania. The selected genotypes were studied from the point of view of the nutritional characteristics of the fruits. Knowledge of the nutritional characteristics of the fruits is important for the selection of biotypes that can be promoted and successfully used in breeding programs, but also in the food and pharmaceutical industries.

MATERIALS AND METHODS

For the study, 100 rose hips from 12 genotypes of *Rosa canina* were collected from the spontaneous flora in the area of the Didactic Station of the University of Craiova, South-West Romania (44°19′ north latitude and 23°48′ east longitude and an altitude above sea level ranging from 176-190 m (https://banumaracine.ucv.ro). This species is very resistant to environmental variations and grows well on rocky, degraded and nutrient-poor soils. The climate variability in southwest Romania is temperate-continental plain, where summers are hot and dry and winters are mild and short, the average annual temperature is 11-12°C, which ensures favorable conditions for the growth and development of *Rosa canina* plants.

Meteorological data were recorded in the wild rosehip fruit harvesting area during 2021 and 2022 (Table 1).
The fruits were harvested at the optimal stage of ripeness for processing (end of October). Immediately after harvesting, the samples were transported to the laboratory and analyzed for the following biochemical parameters, namely: total dry matter, reducing carbohydrates, vitamin C and total phenols.

Table 1. The climatic conditions in South-West Romania

Month	Average temperature (°C)		The relative humidity of the air (%)		Rainfall (mm)	
	2021	2022	2021	2022	2021	2022
May	16.6	17.5	65	59	52	45
June	+20.3	22.5	72	62	106	60
July	25.6	25,1	55	52	17	36
August	25.3	24.9	50	59	13	64
September	18.6	17.9	54	70	11	110

*https://rp5.ru/Vremea_în_Craiova

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Determination of chemical composition

Dry matter content (%) was determined gravimetrically by drying ground fruit of R. canina to a constant weight at 105°C.
Reducing sugars (%) were extracted in distilled water (1:50 w/V), 60 minutes at 60˚C and assayed colorimetric with 3,5-dinitrosalicylic acid reagent using glucose as standard (Soare al., 2017). At 1 mL extract, 2 mL of 3,5-dinitro-salicylic acid reagent (1 g 3,5- dinitrosalicylic acid dissolved in 20 mL of 2M NaOH, 50 mL of distilled water, and 30 g of Na-K- tartrate and the final volume was made up to 100 mL) was added. The mixture was incubated for 10 min at 100˚C, cooled and the absorbance was measured at 540 nm after color development. D-Glucose was used as standard and the results were expressed in % fresh mass basis.
The titratable acidity was determined by titration with 0.1N sodium hydroxide (NaOH), using phenolphthalein as indicator and expressed as mg citric acid/g fw.
Ascorbic acid was extracted in 2% hydrochloric acid, HCl; 5:50 w/v (Soare et al., 2017). The determination of ascorbic acid is performed from the supernatant with iodometric redox titration in which iodine reacts with ascorbic acid, oxidizing it to dehydroascorbic acid. The ascorbic acid content was expressed as mg/g fresh weight.
Methanolic extract: For the determination of phenols and antioxidant activity the samples

were extracted with 80% aqueous methanol (1:10 w:v) by sonicating for 60 min in a sonicate bath Fungilab (Madrid, Spain) equipped with a digital timer and a temperature controller at 24°C. The resulting slurries were centrifuged at 10000 g for 10 minutes and the supernatants were analyzed.
The total phenolics content was determined colorimetric at 765 nm by using the Folin-Ciocalteu reagent (Soare et al., 2017). 0.5 mL methanolic extract of the sample is mixed with 0.2 mL Folin Ciocalteu reagent and 1.8 mL distilled water and after 5 minutes 1.5 mL 7.5% Na_2CO_3 is added. The mixture is left to stand in the dark and the absorbance at 765 nm is read after 60 min. The total phenolic content (TPC) was calculated using a standard curve prepared using gallic acid and expressed as mg GAE/g fw. ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging activity was measured colorimetric at 734 nm using Trolox as standard (Soare et al., 2017). ABTS was dissolved in water to a 7 mmol/L concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS radical cation solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. 0.1 mL of the sample extract was mixed with 2.9 mL of diluted ABTS radical cation solution. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of ABTS radical cation scavenging activity. The final results were expressed as µM TE/g fw.
All spectrophotometric measurements were performed with a Thermo Scientific Evolution 600 UV-Vis spectrophotometer with VISION PRO software. All determinations were performed in triplicate.

Statistical Analysis

The data obtained were analyzed, and all results were expressed as means. The statistical significance of differences between variants was determined with the analysis of variance (ANOVA: single factor), and the means were compared using Duncan's multiple range test ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

The degree of maturity and the harvest period of the rose hips significantly determine their biochemical composition profile. Within these genotypes, the total dry matter recorded a variation amplitude between 31.54 and 46.45%. The highest content was recorded in genotype 10 and was followed by genotypes G4, G5, G6, and G8, while genotypes G1, G7, and G12 recorded lower values. In this case, it can be stated that the dry matter in rose hips from spontaneous flora varies depending on location and genotype. Paunović et al., (2019), reported dry matter content values of 52.28% in fresh fruits and up to 90.33% in dried fruits.

Table 2. Biochemical content of Rosa canina genotypes from the spontaneous flora

Rosehip genotypes	Dry matter content (%)	The titratable acidity (mg citric acid/g fw)	Reducing sugars (%)
G1	31.98f	42.1a	19.2c
G2	35.92^{e}	24.0^{e}	18.0^{cd}
G3	38.30^{d}	31.68^{b}	18.58^{c}
G4	41.12^{c}	25.6^{d}	19.82^{b}
G5	$42.28b^{c}$	26.24^{d}	20.75^{b}
G6	$42.26b^{c}$	30.72^{bc}	23.06^{a}
G7	31.54^{f}	29.44^{c}	20.53^{b}
G8	42.88^{b}	28.16^{cd}	17.19^{d}
G9	39.12^{d}	25.6^{d}	18.29^{cd}
G10	46.45^{a}	40.32^{a}	23.32^{a}
G11	35.44^{e}	29.44^{c}	16.69^{d}
G12	31.90^{f}	21.76^{f}	14.09^{e}
$p \leq 0.05$	1.64	2.05	1.31

Mean values with different superscript letters significantly differ, and statistical significance was set at $p < 0.05$.

Sugars and organic acids are the most common soluble constituents of fruits, with important influence on taste and nutritional properties (Agagik et al., 2020).
Reducing carbohydrates in *Rosa canina* fruits ranged between 14.06 and 23.32% (Table 2). In the specialized literature, a wide variability of carbohydrate content has been reported, which is influenced by genotype, altitude or degree of fruit ripening. Thus, Rosu et al. (2011), reported the total sugars content in the fruits of rose species of 17.63 g/100 g fw to 12.05 g/100 g fw, and Gunes & Dolek (2024) reported values from 9.28-13.90%.
Also, the acidity of fresh *Rosa canina* fruits recorded values from 21.76 to 42.1 mg citric

acid/g fw. These results are in agreement with the studies of Vasilj et al. (2024), which reported values up to 45.1% total acids, and higher than those reported by Güneş et al. (2016), which ranged from 11.1 to 36.7 g kg^{-1} citric acid, influenced by the harvest period.

Antioxidants play an important role in the body in defense mechanisms against pathologies related to free radical attack.

The ascorbic acid (vitamin C) content identified in the rose hip genotypes ranged from 2.64 to 7.04 mg/g f.w. (Figure 1). The influence of genotype on the ascorbic acid content of rose hips recorded in the present study is also confirmed by other authors. Similar results to our study were reported by Peña et al. (2023) who reported values of vitamin C content (the ascorbic acid concentration ranged from 6.0 to 8.2 mg g^{-1} FW).

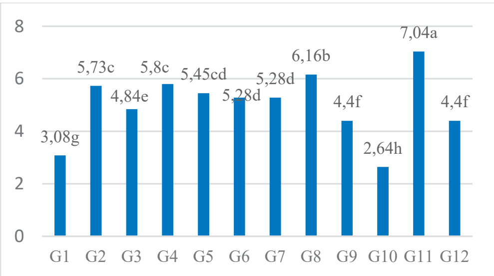

Figure 1. The vitamin C content (mg/g fw) of *Rosa canina* genotypes from the spontaneous flora ($p \leq 0.05 = 0.35$)

Some authors have stated that the vitamin C content of rose hips can also be influenced by the species. Thus, the highest vitamin C content was found in *R. rugosa* and *R. rubiginosa* of 20.9 and 13.9 mg/g dry matter, respectively, while the lower content of ascorbic acid was found in *R. micrantha* (6.1 mg/g dry matter) (Guantaroi et al., 2023). Also, Bakhtiar et al. (2023) reported a vitamin C content of 103.51 and 419.70.12 mg/100 g DW in the fruits of *Rosa canina* ecotypes, collected from eight provinces of Iran, and Bozhuyuk et al. (2021) reported a content of rose hip ecotype located in the East Anatolia of Turkey, ranging from 430-690 mg per 100 g FW (fresh weight).

In a comparative study conducted by Kerasioti, et al. (2019) on species of the Rosaceae family collected from Greece, the variation in antioxidant activity determined by the DPPH test on rosehip fruits was as follows: *Rosa canina* (100 µg/mL) > *Rosa sempervivens* (130 µg/mL) > *Pyracantha coccinea* (500 µg/mL).

In Romania, in the north-eastern part (400-1060 m altitude) Rosu et al. (2011) found vitamin C between 616 and 866 mg/100 g FW in fruits of *Rosa caesia* and *R. rubiginosa* in Romania, and Roman et al. (2013), between 112 and 360 mg per 100 g of fresh rose hips.

Ascorbic acid, phenolic compounds and flavonoids contribute to the antioxidant activities of fruits and, with higher content, fruits exhibit stronger antioxidant activities (Miljković et al., 2024).

The significant differences recorded in the content of phenolic total in fresh rosehip fruits (Figure 2) can be explained by genetic variation between genotypes and the influence of environmental factors in the growing areas.

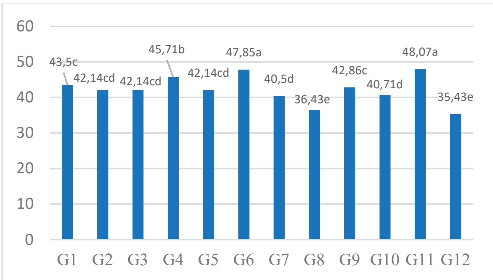

Figure 2. The total phenolic content (mg GAE/g fw) of *Rosa canina* fruits ($p \leq 0.05 = 1.81$)

Munteanu et al. (2023) argue that phenolic compounds are often produced in response to the stress of environmental factors, such as exposure to UV radiation, temperature, humidity and soil nutrient availability. Geographic location also plays an important role, contributing through specific microclimate and soil characteristics. For example, research conducted prior to this study shows that regional variations influence phenolic content in similar plants due to adaptation to local stress (Giosanu et al., 2018; Giura et al., 2019). Therefore, genotypes from the wild flora can accumulate varying amounts of phenolic compounds to better adapt to local conditions. This complex interaction between genetics and environment is essential for the variability observed in the phenolic composition of fruits. In the present study, the content of total phenolic compounds ranged from 35.43 to 48.07 mg GAE/g fw

(Figure 2). The values obtained in this study are comparable to those reported by other authors, where the highest and lowest levels of total phenolics in rose hips were 52.87 and 104.52 mg GAE/g DW (Bakhtiar et al., 2023) or between 390 and 532 mg gallic acid equivalent per 100 g FW (Bozhuyuk et al., 2021), and Soare et al. (2015) reported values of up to 48.07 mg GAE/kg^{-1} in Romania (Oltenia).

The antioxidant properties of rose hips can be influenced by numerous factors, such as its geographical location, ripening stage (Skrypnik et al., 2019), meteorological conditions (Mitroi et al., 2023). The ABTS values of the analyzed genotypes are presented in Figure 3, and the results indicate significant differences between them, in terms of antioxidant capacities. Thus, the highest results were recorded at G1 (182.48 mg/g), followed by G3 (180.33 µM TE/g fw.) and G11 (179.69 µM TE/g fw.).

Bozhuyuk et al. (2021) reported antioxidant capacity values from *R. canina* fruits ranging from 19.7 mg ascorbic acid equivalent per g FW to 34.7 mg ascorbic acid equivalent per g FW, depending on the ecotype. Bünyamin et al. (2021) reported values between 39,510 and 72,673 mmol kg^{-1} for *R. canina* fruits collected from the natural flora of Mesudiye (Ordu) and Talas (Kayseri) districts, Turkey, and Demir et al. (2014) reported values of 35.51 mmol TE g^{-1} by the ABTS method. Differences between studies may be attributed to different species, ecotypes or extraction methods.

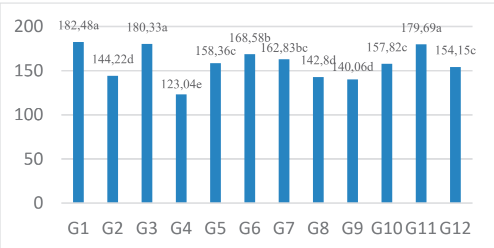

Figure 3. The antioxidant activity (µM TE/g fw) of *Rosa canina* ($p \leq 0.05 = 6.76$)

According to data from scientific literature, the concentration of nutrients in rose hips can be influenced by several parameters, namely: average temperature in the researched area, amount of precipitation during the growing season, species, genotype and altitude, but also by the time of harvest.

CONCLUSIONS

Evaluating the recorded results, it is found that the genotype has the greatest impact on the concentration of nutraceutical compounds. Within the 12 genotypes, a large variation in nutritional values can be observed regardless of the type of compound analyzed. The rosehip genotypes collected from the spontaneous forest in south-western Romania have proven to be rich in bioactive compounds and have the potential to be introduced into germplasm collections. Their analysis indicates that the genotypes presented large variations regarding the content of reducing carbohydrates, vitamin C, antioxidant activity and total phenols. The nutraceutical value of the *Rosa canina* species is undeniable and represents an important source of vitamins and other nutrients very necessary for the human body, which is why the consumption of these fruits can only help in the prophylaxis and prevention of various pathologies.

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