Content of biologically active substances and antioxidant activity of aqueous-ethanol extracts of cultivated rosehip fruit obtained by ultrasonic extraction

Ira Taneva[1], *Nadezhda* Mazova[2], *Iliana* Nikolova[3*], and *Martina* Pencheva[4]

[1]Trakia University, Stara Zagora, Faculty of Technics and Technology, 8600 Yambol, Bulgaria
[2]University of Food Technologies, 4002 Plovdiv, Bulgaria
[3]University of Ruse "Angel Kanchev", Branch-Razgrad, 7200 Razgrad, Bulgaria.
[4]University of Ruse "Angel Kanchev", 7017 Ruse, Bulgaria

Abstract. The aim of the current work is to determine the main biologically active substances and antioxidant activity of aqueous-ethanolic extracts of cultivated rosehip fruit obtained by ultrasonic treatment. The extraction was performed under the following technological parameters: ultrasound frequency 37 kHz, solvents 50 and 70% ethanol, raw material: solvent ratio = 1:20, temperature 20, 40 and 60oC, duration 15, 30 and 60 min. Tannins, ascorbic acid and total phenolic contents were determined in the extracts. The highest tannin content (1.90%) was obtained by extraction with 50% ethanol, temperature 60oC and process duration 60 min. The total phenolic content under the different process conditions is comparable. Higher values of total phenols (45.43 mg GAE g-1) were determined at 50% ethanol solvent, 40oC temperature and 60 min process duration. The highest values of antioxidant activity, by the DPPH method, were reported in extracts with 50% ethanol, temperature 40oC and duration 60 min (422.5 µmol TE/g), and by FRAP method under the same extraction conditions - the value was almost twice lower (231.9 µmol TE).

1 Introduction

Since ancient times, people have used herbs and their aqueous extracts in traditional medicine and for flavouring food products due to their rich composition of biologically active substances.

One of the methods of processing plant raw materials is conventional extraction. The extraction process consists of several operations that include pre-processing of the plant material (drying, grinding, storage) and post-processing of the resulting liquid extract (filtration, concentration, purification, *etc*.). Conventional extraction often takes a lot of time and energy, and environmentally harmful solvents are used (methanol, dichloromethane, acetone, etc.). The extracts obtained are not always safe as they contain residues of solvents and other intermediates produced during the process [1, 2].

* Corresponding author: inikolova@uni-ruse.bg

At the end of the twentieth century, the twelve principles of the so-called "green chemistry" were published, which are generally accepted by many researchers in the development of new methods for the extraction of biologically active substances [3]. New innovative methods of extraction of plant raw materials for environmental protection have been introduced, such as ultrasonic extraction, supercritical extraction, microwave extraction and extraction with eutectic solvents [4].

Compared to conventional extraction, the ultrasonic extraction has several advantages, such as less time and less energy consumption, applying low temperatures and preserving the quality of the resulting extracts. In ultrasonic extraction, the destruction of plant tissue is caused by physical forces created during acoustic cavitation, which favours the release of extractable components into the solvent in a much shorter time [5].

Among the medicinal plants rich in biologically active substances of the Rosaceae is the rosehip (*Rosa canina* L.). The fruits are high in phenolic compounds, flavonoids, anthocyanins, phenolic acids and vitamin C [6-8].

The fruits are also rich in vitamins K and P, the mineral elements Mg, Fe, Mn, and Al [9]. The presence of phenolic acids, hydrolysable tannins, proanthocyanidins, anthocyanins as well as carotenoids represented by β-carotene, rubixanthins and lycopene has been found [10].

In Bulgaria, rosehip is found in bushes and grassy slopes, near rivers, plains, and mountains up to 2000 m altitude throughout the country.

The variety "Plovdiv - 1" has been selected from the wild-growing rosehip in our country, and the fruits are characterized by a high vitamin C content (over 8300 mg/100 g) and excellent taste qualities. [11].

There is no data on the processing of rosehip fruits by ultrasonic extraction and their characterisation.

Therefore, the present study aims to obtain aqueous-ethanolic extracts of cultivated rosehip fruit by ultrasonic extraction, to determine the content of the main biologically active substances in them as well as their antioxidant activity with a view to their inclusion in functional foods.

2 Materials and methods

2.1 Plant material

In this study we used dry fruits of cultivated rosehip – variety "Plovdiv-1" from the area of Gotse Delchev (Blagoevgrad, Bulgaria), harvest 2022, purchased from a private grower. The fruits were stored in double paper bags in a wooden cabinet for one year at room temperature, away from direct sunlight and other heat sources. Before analysis, the fruit was ground on a laboratory grinder (Model PRO 02; 2600 rpm/min), separating the seeds from the flesh (husk) beforehand.

By drying at 105℃ the moisture of the fruit was determined.

2.2 Extraction of the fruits

Ground dry rosehip fruits are extracted in an ultrasonic bath - model ELMA, Elmasonic P30H - Germany, with a frequency of 37 kHz. The technological parameters of the process were: solvent 50 and 70% ethanol; hydromodule 1:20; temperature - 20, 40, and 60^{o}C; duration - 15, 30, 60 min.

The process parameters were chosen based on our preliminary studies [12, 13]. The resulting extracts were centrifuged for 10 min at 4000 rpm^{-1} and stored at 4^{o}C until analysis.

2.3 Chemical analyses

The content of tannins [14], vitamin C [15], and total phenolic content [16] were determined in the fruits and extracts obtained.

2.4 Antioxidant activity

The antioxidant activity was determined by two methods: DPPH radical scavenging activity [17] and FRAP assay [18], with the values presented as mM Trolox Equivalent (TE)/g.

2.5 Statistical analysis

Threefold repetition was used during the experiment, which mean values are presented in the results and discussion section. They were obtained using the MicroCal™ Origin software.

3 Results and discussion

Cultivated rosehip fruits are characterized by a moisture content of 11.27%, a tannin content of 13.24%, a vitamin C content of 153.8 mg/100 g and a total polyphenolic content of 8400 mg GAE/g. [19].

The values of the determined biologically active substances in the extracts are presented in Table 1.

Table 1. Content of biologically active substances in aqueous-ethanol extracts of rosehip fruit

Temp., °C	Duration, min	Tannins %		Ascorbic acid, mg/100 g		Total phenolic, mg GAE/g	
		50 % Ethanol	70 % Ethanol	50 % Ethanol	70 % Ethanol	50 % Ethanol	70 % Ethanol
20	15	0.54 ± 0.0	0.62 ± 0.0	10.54 ± 0.10	8.56 ± 0.08	43.52 ± 0.42	41.53 ± 0.40
	30	0.56 ± 0.0	0.65 ± 0.0	20.48 ± 0.20	8.95 ± 0.08	43.55 ± 0.42	41.65 ± 0.40
	60	0.68 ± 0.0	0.68 ± 0.0	25.67 ± 0.24	9.01 ± 0.08	43.81 ± 0.42	41.72 ± 0.40
40	15	0.66 ± 0.0	0.71 ± 0.0	30.25 ± 0.30	25.28 ± 0.24	43.34 ± 0.42	41.23 ± 0.40
	30	0.69 ± 0.0	0.79 ± 0.0	70.55 ± 0.65	45.58 ± 0.42	43.35 ± 0.42	41.38 ± 0.40
	60	1.05 ± 0.01	1.15 ± 0.01	118.20 ± 1.10	89.52 ± 0.85	45.43 ± 0.44	41.24 ± 0.40
60	15	0.85 ± 0.0	0.69 ± 0.0	26.84 ± 0.25	22.36 ± 0.20	42.57 ± 0.41	41.36 ± 0.40
	30	1.56 ± 0.01	0.72 ± 0.0	35.52 ± 0.34	24.45 ± 0.22	43.06 ± 0.41	41.26 ± 0.40
	60	1.90 ± 0.01	1.22 ± 0.01	101.52 ± 10.0	79.52 ± 0.77	43.54 ± 0.42	41.28 ± 0.40

Tannins, along with phenols and ascorbic acid, are chemical elements included in the composition of rosehip that function as antioxidants [20]. Tannins can trap free radicals depending on their molecular weight and the type of aromatic rings [21].

The highest values of tannins (1.90%) are reported in extracts with 50% ethanol, temperature 60°C and duration 60 min. With the same extraction parameters, the tannin contents in extracts with 70% ethanol were 1.22%.

The data show that the highest ascorbic acid content (118.20 mg/100 g) was found at 50% ethanol, at 40°C and a process duration of 60 min. For comparison, for extracts with 70% ethanol, the content of ascorbic acid is 89.52 mg/100 g. As the temperature increases, the ascorbic acid content decreases, which is explained by its thermolability.

The total phenolic content at the different technological parameters is comparable. Higher values (45.43 mg GAE/g) are reported at 50% ethanol, 40°C temperature and 60 min duration. In the extracts obtained with 70% ethanol at the same process parameters, the total phenolic content is 41.24 mg GAE/g.

The total phenolic content values in the extracts obtained are comparable to those reported by other authors (52.94 mg/g and 31.08 mg/g) [22, 23].

Phenolic acids have been found to have different biological properties [24].

Some authors found higher values for total phenolic content in aqueous extracts obtained from fruits of different rosehip species from Turkey (9600 mg GAE/100 g DW) [25] and (3108 mg GAE/100 g DW) [22], as well as for extracts with methanol (from 5030 to 6100 mg GAE/100g) [26].

From the analyses performed, it can be summarized that ultrasonic extraction is suitable for obtaining extracts enriched in biologically active substances at the following technological parameters: solvent – 50% ethanol, temperature – 40ºC and duration – 60 min.

In Fig. 1 and Fig. 2 the values of the determined antioxidant activity are presented. Data show that extracts with 50% ethanol have a higher antioxidant capacity than extracts with 70% ethanol.

The antioxidant activity of the extracts measured by the FRAP method has lower values compared to those obtained by the DPPH method.

The highest values of antioxidant activity by the DPPH method were reported for extracts with 50% ethanol, temperature 40ºC and duration 60 min (422.5 µmol TE/g), and by the FRAP method under the same extraction conditions (231.9 µmol TE/g).

Various studies prove that the antioxidant activity of plant extracts is in positive correlation with bioactive compounds [27, 28].

The antioxidant activity values obtained in the extracts by the FRAP method are comparable with the data of other authors [22, 29, 30], but are much higher than those of other authors [31].

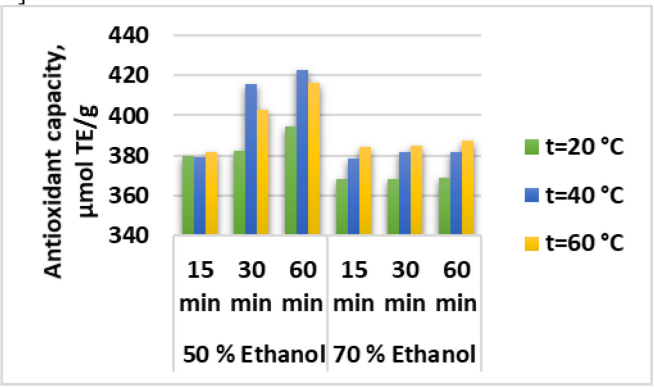

Fig. 1. Antioxidant activity (DHHP) of aqueous-ethanol extracts of cultivated rosehip fruit.

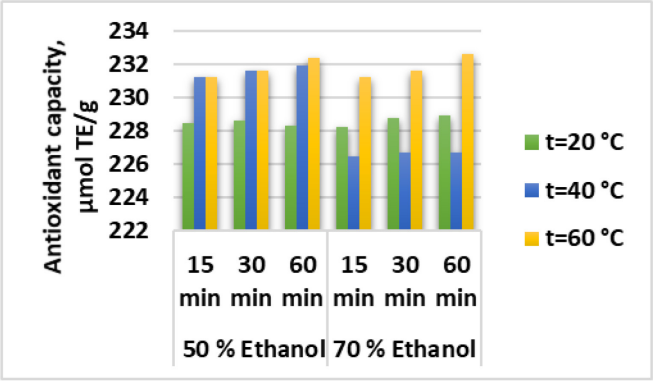

Fig. 2. Antioxidant activity (FRAP) of aqueous-ethanol extracts of cultivated rosehip fruit.

The main criterion for the evaluation of the relationship between the studied biologically active substances (tannins, total phenols and ascorbic acid) and antioxidant activity is the correlation coefficient (R^2). It offers information on the strength of the relationship between the variables – biologically active substances and antioxidant activity.

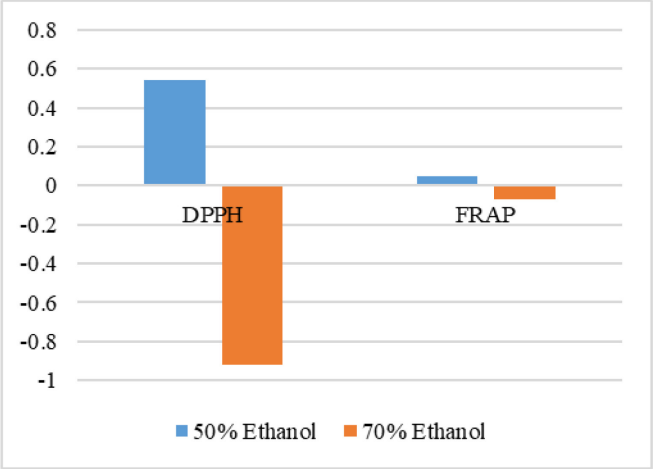

Fig. 3. Correlation between total phenolic compounds and antioxidant activity in aqueous-ethanol extracts of cultivated rosehip fruit.

From the data presented in Fig. 3, there is a correlation between total phenolic content and antioxidant activity in the extracts with 50% ethanol. This correlation is more pronounced when antioxidant activity is determined by the DPHH method with 50% ethanol ($R^2 = 0.61$), suggesting that the parameters may significantly influence each other.

A similar correlation between the total phenolic content and antioxidant activity of fruits of different species of rosehip (*Rosa canina* and *R. rubiginosa* L.*)* was also established by other authors [32].

4 Conclusions

Aqueous-ethanol extracts of cultivated rosehip fruit variety "Plovdiv-1" obtained by ultrasonic extraction are a potential source of various biologically active substances that are related to the measured antioxidant activity.

This can serve as a basis for future research - on the inclusion of the aqueous - ethanol rosehip extracts in a variety of new functional foods for benefiting the human health.

References

1. J. Azmir, I. Zaidul, M. Rahman, K. Sharif, A. Mohamed, F. Sahena, M. Jahurul, K. Ghafoor, N. Norulaini, A. Omar, Techniques for extraction of bioactive compounds from plant materials: a review. J. Food Eng. **117**, 426 (2013).
2. B. Kaufmann, P. Christen, Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. Phytochem. Anal. **13**, 105e (2002).
3. P. Anastas, J. Warner, Green chemistry: Theory and practice (Oxford University Press, London, 1998).

4. F. Chemat, M. Abert-Vian, A. Fabiano-Tixier, J. Strube, L. Uhlenbrock, V. Gunjevic, G. Cravotto, Green extraction of natural products. Origins, current status, and future challenges. Trends Analy. Chem. **118**, 248 (2019).
5. M. Ashokkumar, Applications of ultrasound in food and bioprocessing. Ultrason. Sonochem. **25**, 17 (2015).
6. C. Chrubasik, R. Duke, S. Chrubasik, The evidence for clinical efficacy of rose hip and seed: a systematic review. Phytother Res. **20**, 1 (2006).
7. R. Soare, D. Bonea, P. Iancu, M. Niculescu, Biochemical and technological properties of *Rosa canina* L. fruits from spontaneous flora of Oltenia, Romania. Bull. Univ. Agric. Sci. Vet. Med. Cluj-Napoca. Hortic. **2**, 182 (2015).
8. Q. Yang, D. Zhang, A. Farha, X. Yang, H. Li, K.-W. Kong, J.-R. Zhang, C.-L. Chan, W.-Y. Lu, H. Corke, R.-Y. Gan, Phytochemicals, essential oils, and bioactivities of an underutilized wild fruit Cili (*Rosa roxburghii*). Ind. Crops Prod. **143**, 111928 (2020).
9. N. Montazeri, E. Baher, F. Mirzajani, Z. Barami, S. Yousefian, Phytochemical contents and biological activities of *Rosa canina* fruit from Iran. J. Med. Plants Res. **5**, 4584 (2011).
10. G. Angelov, S. Boyadzhieva, S. Georgieva, Rosehip extraction: Process optimization and antioxidant capacity of extracts. Centr. Eur. J. Chem. **12**, 502 (2014).
11. M. Popova, Hawtorn – Perspective culture of agriculture (Center for European Integration and Culture, at Higher Education University "Agricultural College", Plovdiv, 2009).
12. I. Taneva, K. Dobreva, Study on the composition of rosehip fruit extracts (*Rosa canina* L.). Sci Works UFT, **60** (2013).
13. I. Taneva, T. Chalakova, K. Dobreva, Study on the composition of ethanol extracts of rosehip fruits, Int. Sci. Conf. "Technology, Technologies and Education" Proceedings, October 30-31, Yambol (2013).
14. State Pharmacopoeia of the USSR, XI, (Publishing house "Medicine", Moscow, 1990).
15. Association of Official Analytical Chemists, Vitamin C in juices and vitamin preparations, Official Method 967.21, In: AOAC Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA, 45.1.14 (2005).
16. I. Ivanov, R. Vrancheva, A. Marchev, N. Petkova, I. Aneva, P. Denev, V. Georgiev, A.. Pavlov, Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species. Int. J. Curr. Microbiol. Appl. Sci. **3**, 296 (2014).
17. T. Misharina, Antiradical properties of essential oils and extracts from coriander, cardamom, white red, and black peppers, Appl. Biochem. Microb. **52**, 79 (2016).
18. I. Benzie, J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP Assay. Anal. Biochem. **239**, 70 (1996).
19. M. Ivanova, I. Taneva, M. Zhekova-Kalaydzhieva, M. Schreiner, A. Slavov, Y. Tumbarski, Comparative analysis of wild and cultivated rosehip from Bulgaria. Annals Un Dunarea de Jos of Galati Fascicle VI – Food Technol. **47**, 140 (2023).
20. M. Ahmad, Z. Kaloo, B. Ganai, H. Ganaie, S. Singh, Phytochemical screening of *Meconopsis aculeata* royle an important medicinal plant of Kashmir Himalaya: A perspective. Res. J. Phytochem. **10**, 1 (2016).
21. D. Samec, M. Maretic, I. Lugaric, A. Mesic, B. Salopek-Sondi, B. Duralija, Assessment of the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using principal component analysis. Food Chem., **194**, 828 (2016).

22. N. Demir, O. Yildiz, M. Alpaslan, A. Hayaloglu, Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (*Rosa* L.) fruits in Turkey. LWT-Food Sci. Technol. **57**, 126 (2014).
23. G. Fascella, F. D'Angiolillo, M. Mammano, M. Amenta, F. Romeo, P. Rapisarda, G. Ballistreri, Bioactive compounds and antioxidant activity of four rose hip species from spontaneous Sicilian flora. Food Chem., **289**, 56 (2019).
24. H. Gazwi, Preventive effect of lemongrass (*Cymbopogon citratus*) against oxidation in soybean oil. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci*,* **90**, 151 (2020).
25. S. Ercisli, Chemical composition of fruits in some rose (*Rosa* spp.) species. Food Chem. **104**, 1379 (2007).
26. A. Najda, H. Buczkowska, Morphological and chemical characteristics of fruits of selected *Rosa* sp. Mod. Phytomorph. **3**, 99 (2013).
27. S. Sinha, M. Kumar, A. Kumar, S. Bharti, V. Shahi, Antioxidant activitiy of different tissue extract of faba bean (*Vicia fabal*) containing phenolic compounds. Legume Res. **36**, 6, 496 (2013).
28. P. Beniwal, S. Jood, Total phenolic content and antioxidant activity of by-products from cereal and legume milling industries. Asian J. Dairy and Food Res. **33**, 307 (2014).
29. X. Gao, L. Björk, V. Trajkovski, M. Uggla, Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. J. Sci. Food Agric. **80**, 2021 (2000).
30. J. Nadpal, M. Lesjak, F. Šibul, G. Anackov, D. Cetojevic-Simin., N. Mimica-Dukic, I. Beara, Comparative study of biological activities and phytochemical composition of two rose hips and their preserves: *Rosa canina* L. and *Rosa arvensis* Huds. Food Chem. **192**, 907 (2016).
31. I. Koca, N. Ustun, T. Koyuncu, Effect of drying conditions on antioxidant properties of rosehip fruits (*Rosa canina* sp.). Asian J. Chem. **21**, 1061 (2009).
32. F. Peña, S. Valencia, G. Tereucán, J. Nahuelcura, F. Jiménez-Aspee, P. Cornejo, A. Ruiz, Bioactive compounds and antioxidant activity in the fruit of rosehip (*Rosa canina* L. and *Rosa rubiginosa* L.). Molecules. **28**, 3544 (2023).