SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(4) (2023) 323-332, DOI: 10.24323/akademik-gida.1422139

Research Paper / Araştırma Makalesi

Mineral and Bioactive Component Contents of Rosehip (*Rosa canina* L.) Seed Powder

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Received (Geliş Tarihi): 01.10.2023, Accepted (Kabul Tarihi): 25.12.2023
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ABSTRACT

This study determined the bioactive components of rosehip (*Rosa canina L.*) extract obtained via supercritical carbon dioxide extraction. The total phenolic content of its extract was 214.4 mg gallic acid equivalent/kg, with the total flavonoid content of 21.1 mg quercetin equivalent/kg. The antioxidant activity of the extract, which was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, was 64.8 µmol Trolox® equivalent antioxidant capacity (TEAC)/g. Gas chromatography-mass spectrometry identified 15 bioactive components in the extract. Additionally, pre- and post-processing heavy metal analyses were conducted on rosehip powder and seeds using inductively coupled plasma mass spectrometry (ICP-MS). Results showed that metal concentrations ranged from 0.064 to 9134.1 mg/kg in rosehip powder and from 0.143 to 1929.5 mg/kg in seeds, with the concentrations of potassium and magnesium as the highest. Despite the limited uses of rosehip products, this study indicated that wild rosehips are rich in functional components with potential health benefits.

Keywords: Rosehip powder, *Rosa canina* L., Heavy metal, Supercritical carbon dioxide extraction, Bioactive component

Kuşburnu (*Rosa canina* L.) Tohumu Tozunun Mineral ve Biyoaktif Bileşen İçerikleri

ÖZ

Bu çalışma, süperkritik karbon dioksit ekstraksiyonu yoluyla elde edilen kuşburnu (*Rosa canina* L.) ekstraktının biyoaktif bileşenlerinin analizine odaklanmıştır. Toplam fenolik içeriğin 214.4 mg gallik asit eşdeğeri/kg, toplam flavonoid içeriğinin ise 21.1 mg kuersetin eşdeğeri/kg olduğu bulunmuştur. DPPH (2,2-difenil-1-pikrilhidrazil) yöntemi ile belirlenen antioksidan aktivitenin 64.8 µmol TEAC/g olduğu tespit edilmiştir. Gaz kromatografisi kütle spektrometresi, özütte 15 biyoaktif bileşeni tanımlamıştır. Ayrıca kuşburnu tozu ve tohumları üzerinde, indüktif eşleşmiş plazma kütle spektrometresi (ICP-MS) kullanılarak işlem öncesi ve sonrası ağır metal analizleri yapılmıştır. Sonuçlar, kuşburnu tozunda 0.064 ila 9134.1 mg/kg ve tohumlarda 0.143 ila 1929.5 mg/kg arasında değişen konsantrasyonları göstermiştir, potasyum ve magnezyum en yüksek konsantrasyonları göstermiştir. Çalışma yabani kuşburnunun potansiyel sağlık faydalarına sahip fonksiyonel bileşenler açısından zengin olduğunu vurgulamaktadır.

Anahtar Kelimeler: Kuşburnu tozu, *Rosa canina* L., Ağır metal, Süperkritik karbondioksit ekstraksiyonu, Biyoaktif bileşen

INTRODUCTION

Rosa canina L., commonly known as rosehip or dog rose, is the fruit of plants belonging to the *Rosaceae* family, specifically the *Rosaideae* subfamily. The term "rosehip" refers to the fruit of the rose plant. In Latin, it is referred to as Fructus Rosae [1]. Globally, there are more than 100 species of rosehip distributed across different geographic regions of Europe, Asia, the Middle East, and North America [2]. Turkey, specifically, is home to 27 of these species. Additionally, rosehip includes 5 subspecies, 2 varieties, and 15 hybrids. Sixteen of these rosehip species are particularly located in the Eastern Black Sea region [3].

The utilization of rosehip throughout history underscores its cultural and historical importance. In Mediterranean countries during ancient times, rosehip was used as a symbol of purity and cleanliness. The Romans, for instance, utilized rosehip flowers as medicine for abdominal pain and made wine, jam, and cakes from their fruits. Throughout history, rosehip has been a valuable plant used for various purposes in different cultures. The recognition of rosehip as an edible natural resource further emphasizes its significance within the broader context of non-wood forest products.

The rosehip fruit is one of the richest fruits in nature, especially in terms of anthocyanins, proanthocyanidins, catechins, quercetin, gallic acid, ellagic acid, flavonoids, and other polyphenolic compounds. Additionally, it contains essential nutrients such as organic acids, essential fatty acids (omega-3 and omega-6), tocopherols (a form of vitamin E), carotenoids (precursors of vitamin A), vitamin C, phenolics, and sugars [4]. These components collectively contribute to the antioxidant [5], anti-inflammatory [6], antiproliferative [7], anti-obesity, anti-diabetic activity [8], and overall positive health effects of rosehip. Rosehips also provide other essential vitamins and minerals, including vitamin P, K, E, B1, B2, provitamin A, calcium, zinc, potassium, iron, magnesium, manganese, sodium, and phosphorus, making them a highly beneficial fruit for overall health [9].

During the processing of rosehips, the pulp of the rosehip is the main utilized part, while the remaining seeds are generally considered as waste. However, the seeds also have significant applications. The economic value of rosehips, coupled with the soothing properties of the seeds, enhances their importance. Additionally, an experiment conducted with mice fed with rosehip seeds has raised the possibility of using rosehip seeds as a component in dietary human foods [10,11]. Rosehip seeds are abundant in unsaturated fatty acids (FAME's) and boast a higher vitamin E content when compared to rosehip pulp. They serve as an excellent source of omega-6 FAME's and exhibit significant antioxidant capacity [12]. The most abundant FAME's found in the seed oil are linoleic acid (50.08%), arachidic acid (20.00%), and oleic acid (19.31%) [11,13].

The nutritional richness of rosehip, coupled with its versatility in culinary applications and potential health-promoting properties, makes it a valuable resource. However, processing and consumption methods can influence its content levels. An ideal extraction method should be fast, simple, and cost-effective, and no additional steps should be required to prepare it for analysis. Traditional extraction methods involve the use of exhaustive solvent extraction for long periods. While these methods yield high amounts of analytes, they also involve the overuse of harmful solvents, which can violate environmental and health guidelines. For this reason, there is an urgency to develop cost-effective and environmentally friendly extraction processes that may compete advantageously with the industrially established method. These processes should preserve the nutritional, functional, and biological properties of analytes while ensuring high extraction yields.

Among these alternative processes, supercritical fluid extraction (SCFE), which was developed in the mid-1980s, has been suggested as a promising solution to the challenges in the extraction process. This process is highly effective due to the low polarity of CO_2, which is ideal for extracting non-polar compounds such as essential oils, FAME's, tocopherols, and carotenoids from plant material. This method eliminates the need for hazardous organic solvents and prevents thermal degradation of thermosensitive compounds. Supercritical fluids have the unique ability to diffuse into solid matrices like a gas while simultaneously solubilizing certain compounds of interest in the solid material. The utilization of carbon dioxide in SFE-CO_2 provides additional benefits, given its affordability, non-toxicity, recyclability, chemically inert, non-inflammability, abundant availability, and non-polar nature. Moreover, the efficient separation of CO_2 through depressurization at the end of the process enhances overall extraction efficiency [14].

This study evaluated the total phenolic content, total flavonoid content, and antioxidant activity of an extract made from rosehip powder using the Supercritical Carbon Dioxide Extraction (SFC-CO_2) method. In addition, a gas chromatography-mass spectrometry (GC-MS) examination was conducted to determine whether the extract contained any bioactive ingredients. This study also investigates the fruit's heavy metal contents both before and after the extraction process.

MATERIALS and METHODS

Plant Material

Commercially purchased rosehip flour, labelled as 100%, was stored in its original packaging in a refrigerator set at 4°C until used in the experiment

Chemicals

Methanol (CH_3OH, purity>99.8%), heptane (HPLC, ≥99%), and hexane (C_6H_{14}, purity>99.99%) were purchased from IsoLab (Eschau, Germany). Additionally, high-purity gallic acid, Folin-Ciocalteu reagent, DPPH, Trolox (±-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium chloride,

boron trifluoride and sodium carbonate were obtained from Sigma Aldrich. Solutions were prepared and diluted using ultra-pure deionized water with a resistance of 18.2 MΩ cm^{-1} that was acquired from a reverse osmosis system (Human Corp. Seoul, South Korea).

Apparatus

All weighing in the experiments were performed on an analytical balance with a sensitivity of 0.1 mg (Denver Instrument, APX-200 model, USA). The mixing processes were carried out using a vortex device (Velp Scientifica, ZX Classic model, Italy). For the separation process, a centrifuge device (EBA 20, Hettich, Zentrifugen, Tuttlingen, Germany), was used. Laboratory materials and extractor bags were dried using a hot air sterilizer (Nüve, FN 055 Model, Istanbul, Turkey). A UV-Vis spectrophotometer with 8 cells (EMC-11, Duisburg, Germany) was used to determine the total phenol contents.

Supercritical Fluid Extraction

After placing 150 g of ground rosehip powder (Figure 1a) into special extraction bags (Figure 1b), they were loaded into the extraction cell. The extraction took place in a 0.5 L stainless steel vessel (Figure 1c). Optimal extraction conditions were determined to be as follows: 40°C extractor temperature, 120°C restrictor temperature, 50°C separator temperature, 300 bar pressure (using CO_2 for pressurization),CO_2 flow rate of 50 g/min and a working time of 180 minutes, including a 20 minute static extraction time. The extracted rosehip powder after the extraction process is shown in (Figure 1d). Finally, the extract collected in the vial after extraction (Figure 1e) was stored at +4°C for the determination of antioxidant capacity, total phenolic content, total flavonoid content, and bioactive component quantities.

The efficiency of the supercritical fluid extraction process for rosehip powder is defined as the yield, expressed as a percentage (% yield), calculated by dividing the mass of the extract (g extract) by the mass of the initial dried powder (g dry powder). The amount of phenolic extracts obtained at the end of the extraction process was found to be 3.5% using the following equation;

$$\%Yield = \frac{m_{extract}}{m_{initial\,powder}} \cdot 100 \qquad (1)$$

Where; $m_{extract}$ is the amount of extract (g), and $m_{initial\ powder}$ is the initial amount of dry rosehip powder (g).

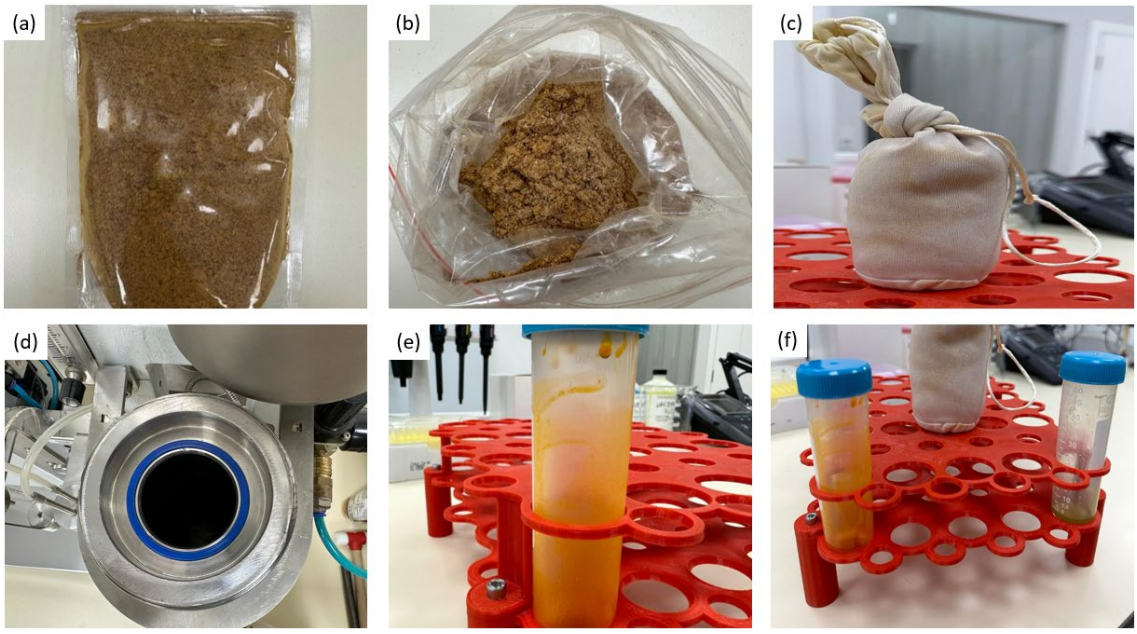

Figure 1. a) Rosehip powder. b) Rosehip powder after the extraction. c) Extraction sac. d) Extraction cell. e,f) Extract collected in vial

FAME's Extraction Method

Transesterification was carried out by boiling 0.1 g of rosehip SFE extract in ca. 4 mL of methanolic KOH for several minutes. Methanolic BF3 (14% w/v, 5 mL) was then added, and the mixture boiled further for 2 minutes. Finally, 5 mL of heptane was added, the vial was shaken and the mixture boiled for 1 minute. To a cooled solution, 15 mL of saturated NaCl (aq) was added and the vial was shaken vigorously (1 min). A 1 mL aliquot of the supernatant heptane layer was transferred into a GC vial for analysis.

DPPH Radical Scavenging Assay

The DPPH· radical scavenging activity of rosehip powder was determined using the DPPH· antioxidant activity method reported in [15]. DPPH· solution is prepared using methanol, and its absorbance is adjusted to 1.1 at 515 nm. A mixture of 150 µL of the sample and 2850 µL of DPPH· is prepared and left in dark environment at room temperature for 1 hour. After the incubation period, the change in absorbance of the samples is analyzed at 515 nm using a UV spectrometer. The average of triplicates was calculated and the obtained values were expressed in mmol Trolox® equivalent (mmol TE) per 100 mL based on the equation derived from the Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard curve. The antioxidant capacity of the samples is determined using the calibration curve ($y = 0.0132x + 0.3419$, $R^2 = 0.9821$) obtained from Trolox® standard solutions (10, 20, 30, 40, and 50 µL).

Total Phenolic Content

The total phenolic content of rosehip powder extract was determined using the Folin-Ciocalteu method, as reported by Singleton et al. [16]. This method is based on a redox reaction where phenolic compounds reduce the Folin-Ciocalteu reagent and convert it to an oxidized form. The steps of the method are as follows. 100 µL is drawn from the prepared extract, followed by the addition of 4.5 mL of distilled water. Subsequently, in the second phase, 100 µL of Folin-Ciocalteu reagent (1 N) is introduced and thoroughly mixed, followed by the addition and thorough mixing of 300 µL of 2% sodium carbonate solution. The resultant mixture is then left in dark environment for a waiting period of 2 hours. Moving on to the fourth step, spectrophotometric analysis is conducted after the waiting period, measuring the absorbance at a wavelength of 760 nm using a UV-visible spectrophotometer. For the blank test, 0.5 mL of methanol is utilized instead of the sample. Finally, under consistent conditions, analysis is carried out using 50-250 mg/L gallic acid standard solutions. The absorbance values obtained are then applied to a calibration curve, and the final result is expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of the sample.

The determination of total phenolic compound content in the samples is expressed in terms of gallic acid equivalents, and this quantification is derived from the calibration curve ($y = 0.0099x + 0.0806$, $R^2 = 0.9999$). The average of three replicated measurements was calculated.

Total Flavonoid Content Analysis

According to the spectroscopic method applied by Zhinsen et al.[17], the total flavonoid content was determined. In this analysis, 1 mL of the sample was taken and transferred to a test tube, and 4 mL of distilled water was added and mixed. Then, 0.3 mL of a 5% $NaNO_2$ solution was added to the tube, and it was homogenized with the help of a vortex. After waiting for 5 minutes, 0.6 mL of a 10% $AlCl_3.6H_2O$ solution was added to the mixture, and after waiting for an additional minute, 2 mL of a 1 mol/L NaOH solution was added. The total volume in the tube was completed to 10 mL with distilled water. The resulting mixture was vortexed once again, and the absorbance value against water was measured at a wavelength of 510 nm. In this way, the total flavonoid content was determined.

The total flavonoid content was calculated based on the quercetin standard curve prepared in concentration intervals with three repeated experiments. The standard curve ($y = 0.0009x + 0.0001$, $R^2 = 0.9975$) was plotted using quercetin solutions of 20, 40, 60, 80, and 100 mg/L. The measurements were made at a wavelength of 510 nm to create the standard curve. Using the generated standard curve, the total flavonoid content in the samples was calculated in terms of milligrams of quercetin per gram of dry weight.

Bioactive Compound Content and FAME's Analysis

For the analysis of the bioactive compound content in the extract, a gas chromatography-mass spectrometer (GC-MS) device was employed. The extract was diluted 50-fold in hexane prior to analysis. The chromatographic system used in this study a Shimadzu QP-2020NX model gas chromatograph-mass spectrometer, incorporating a split-splitless injector system (AOC 20i model autoinjector with AOC 20s automatic sampler). The GC column used was an Rtx-5MS (Restek) column (30 m x 0.25 mm i.d. x 0.25 µm, coated with 95% dimethylpolysiloxane and 5% diphenyl thin film). The carrier gas used was hydrogen with a purity of 99.9999%, generated from ultra-pure water using the Peak Scientific Precision H2 450 model gas generator. The maximum flow rate from the generator is 450 mL/min, and the gas outlet pressure is balanced to 5 bar. The carrier gas (hydrogen) is passed through a molecular sieve and oxygen traps from Shimadzu GLC Ltd. before being sent to the system. All analyses in the device were conducted in the 70 eV electron impact (EI) mode.

The full scan (SCAN) mode was utilized. Following the analysis, qualitative identification was carried out using library scans and similarity indices. GC-MS injector and detector temperatures were 250°C and 300°C, respectively. A split ratio of 10:1 was used to inject 1 µL of sample. Hydrogen carrier gas was used at a flow rate of 1.2 mL min^{-1}. The ion source was kept at 230°C. Solvent delay was set at 1.4 minutes. The column temperature was increased from 40 to 250°C at 3°C min^{-1}.

FAME's were analyzed by GC-FID prior to area normalization-based quantification. A Shimadzu GC-2030 was used. The system was equipped with an Rx-5SilMS column (30 m × 0.25 mm × 0.25 µm). A split ratio of 70:1 was used to inject 1 µL of sample. Hydrogen carrier gas was used at a flow rate of 2.0 mL min-1. The temperature program was as follows: 50°C (1 min hold) to 140°C at 10°C min-1, then to 260°C at 3°C min-1, and held for 5 min. Injector and detector temperatures were 260°C and 280°C, respectively.

Microwave Method

Rosehip powder samples were dissolved using the microwave digestion method. Approximately 0.500 ±0.001 g of rosehip powder is precisely weighed and placed into pressurized Teflon tubes. To aid in the processing, 4 mL of HNO_3 and 1.0 mL of $HClO_4$ are introduced into each tube, followed by sealing with Teflon disks. Subsequently, the tubes were positioned in an oven, and the oven program conditions, set according to the manufacturer's recommendations as outlined in Table 1, were applied. Once the process was complete, the tubes were allowed to cool before being opened under a fume hood. The extracted solutions were then transferred into 50 mL volumetric flasks and the volume was adjusted with distilled water. The resulting solutions for analysis are stored in the refrigerator at +4°C.

Table 1. Microwave oven program

Step	Heating ramp (min)	Time/min	Temperature, °C
1	5	5	145
2	3	5	170
3	2	18	190
4	1	1	75
5	1	1	75

Mineral Content

The mineral content analysis of the rosehip powder sample was conducted at the Advanced Technology Application and Research Center of Pamukkale University. The rosehip fruit, in its non-powdered form, and a commercially purchased sample were simultaneously analyzed through service procurement. For this purpose, both samples were initially dissolved using the CEM brand Mars6 iPrep model microwave dissolution system. The dissolved samples were then analyzed using the Perkin Elmer Nexion2000 model (USA) Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The system uses argon gas as the primary gas and helium as the collision gas for interference removal. With a dual-mode quadrupole, the system has a mass measurement range of 5-279 amu.

RESULTS and DISCUSSION

Total Phenolic Content and Antioxidant Activity

The extract obtained from rosehip powder using the supercritical fluid extraction method was analyzed for its total phenolic content, with concentrations determined using a calibration curve of standard gallic acid solutions ranging from 50 to 250 mg/L ($y = 0.009x + 0.080$, $R^2 = 0.999$). Experimental absorbance values were fitted to this curve to determine their corresponding concentrations. The total phenolic content in the rosehip sample was found to be 214.4 mg GAE/kg.

For the calculation of the total flavonoid content in R. canina powder extractions, the calibration curve provided was $y = 0.000x + 0.0004$, $R^2 = 0.997$. According to the experimental results, the total flavonoid content in rosehip extracts was determined to be 21.1 mg quercetin equivalent/kg.

Calibration curves for antioxidant capacity measurements in rosehip extractions using the DPPH method were given as $y=0.013x + 0.341$, $R^2 = 0.982$. The antioxidant capacity of rosehip extracts, based on DPPH results, was determined to be 64.8 µmol TEAC/kg.

These results indicate that the total phenolic content, flavonoid content, and antioxidant capacities in the fruits of different rosehip species can vary. It was observed that rosehip had a lower total phenolic content compared to other species, but its total flavonoid content and antioxidant capacity were higher.

When examining the results of various extractions of rosehip, as shown in Table 2, it is evident that our research findings align with other studies, indicating that the supercritical carbon dioxide extraction method yields the highest amounts of total phenolic content and total flavonoids in rosehip fruits. However, the antioxidant activity content in supercritical carbon dioxide extraction was found to be lower in comparison to other extractions.

These findings suggest that the choice of extraction method can significantly impact the phytochemical composition of rosehip extracts, highlighting the importance of considering different factors when selecting an extraction method for obtaining specific bioactive compounds.

Bioactive Component Content and FAME's Analysis

In the chromatographic analysis, the sample was directly analyzed without undergoing any preprocessing steps, resulting in the identification of fewer components than expected. The list of these components is provided in Table 3, including their names, CAS numbers, mass spectral information, and similarity index values. Compounds with a similarity index of 70% or higher were identified using the NIST11 (National Institute of Standards and Technology), GCorganic acid, and FFNSC3 (Flavour and Fragrance Natural and Synthetic Compounds) libraries. Figure 2 presents the GC-MS chromatogram of the extract. It was observed that preprocessing steps were necessary for determining the FAME's and sterol composition of the extract. Figure 3 shows the GC-FID chromatogram of FAME's of rosehip seed extract, and the list of these components is provided in Table 4.

Table 2. Total phenolic, total flavonoid, and antioxidant activity content in rosehip powder extract and comparison with the literature

Extraction method	Total phenolic content (mg gallic acid equivalent/kg)	Total flavonoids (mg quercetin equivalent/kg)	Antioxidant (DPPH) activity (mmol Trolox® equivalent/kg)	References
Supercritical carbon dioxide extraction	214.4 ±22.6	2.1±0.2	64.8 ±5.9	This Study
Solid-liquid extraction (50% acetone)	5.09±0.14	-	379±2.81	[18]
Solid-liquid extraction (80% methanol)	2.59±0.14	-	190±4.81	[18]
Solid-liquid extraction (methanol)	424.6±1.8	23.6±4.2	-	[19]
Solid-liquid extraction (aqueous)	74.6±3.08	1.22±0.02	32.7±1.54	[20]
Solid-liquid extraction (methanol)	50.9±3.60	0.65±0.03	21.7±2.04	[20]
Solid-liquid extraction (70% acetone)	21.5±0.33	-	-	[21]
Solid-liquid extraction (90% methanol)	21.2±0.38	-	-	[21]
Solid-liquid extraction (80% ethanol)	16.3±0.31	-	-	[21]
Solid-liquid extraction (methanol)	10.74±3.09	3.43±1.44	25.03±4.91*	[22]
Solid-liquid extraction (methanol+water+formic acid)	31.08±0.19	9.48±0.94	278.90±5.60*	[23]
Solid-liquid extraction (methanol+1% HCL)	6.298±116.7	-	-	[24]
Solid-liquid extraction (methanol)	225.65±2.50	2.02±0.03	-	[25]
Solid-liquid extraction (ethanol)	76.26	-	457.2 ±626.2	[26]
Supercritical carbon dioxide extraction (ethanol)	118 ±13.7	-	-	[27]

* All values are in mg/mL

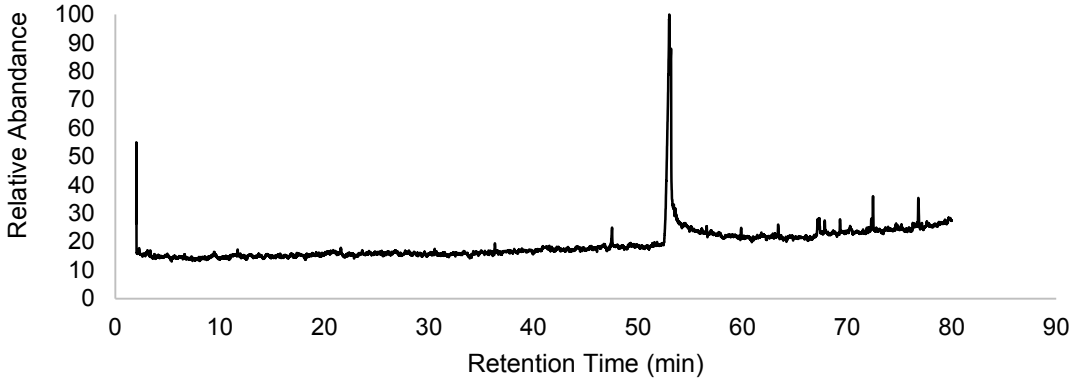

Figure 2. GC-MS chromatogram of bioactive component of rosehip seed extract

Table 3. Bioactive components in rosehip flour extract: GC–MS analysis

#	Compound	t_R	CAS Number	%Area	%Height	Similarity index
1	n-Hexadecanoic acid (palmitic acid)	47.51	57-10-3	4.03	1.42	93
2	Linoleic acid	52.97	60-33-3	14.77	56.91	95
3	Mentha-1(7),8-diene	53.09	13837-95-1	4.34	13.42	81
4	Oleic acid	53.15	112-80-1		11.1	91
5	Hexadec-(11E)-en-1-ol	56.54	61301-56-2	4.13	0.53	85
6	Bis(2-ethylhexyl) adipate	59.86	103-23-1	3.95	0.76	78
7	Pelargol	63.40	106-21-8	4.0	0.89	75
8	9,12-Octadecadienoic acid (Z,Z)- (2-hydroxy-1-(hydroxymethyl)ethyl ester)	67.18	3443-82-1	5.52	1.65	87
9	Tetradec-(7Z)-enal	67.31	65128-96-3	5.69	1.69	84
10	Hexacosane	67.84	630-01-3	3.87	0.91	92
11	9-Octadecenoic acid (Z), methyl ester	69.31	301-02-0	3.15	0.81	88
12	Eicosene	72.31	27400-78-8	4.51	0.99	92
13	Hexacosane	72.47	630-01-3	3.57	2.31	97
14	Tricos-(9Z)-ene	76.28	27519-02-4	5.00	0.65	79
15	Dotriacontane	76.80	544-85-4	4.74	2.64	92

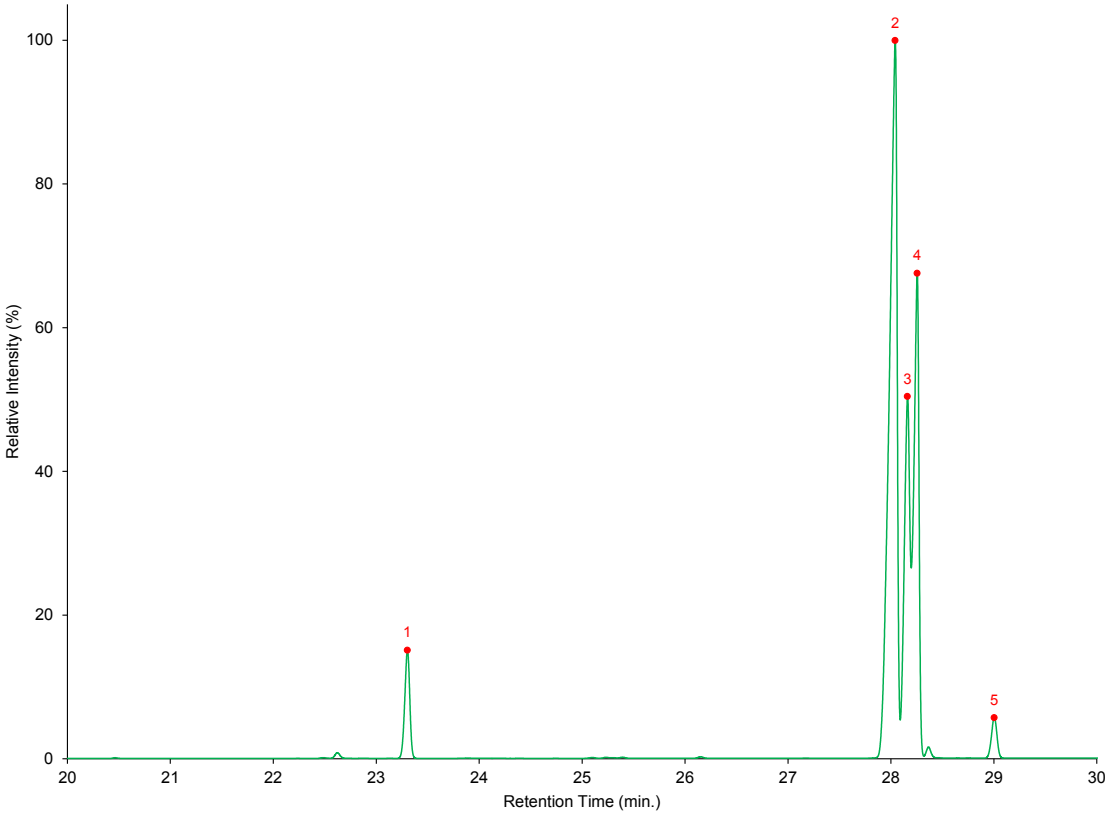

Figure 3. GC-FID chromatogram of FAME's of rosehip seed extract

Table 4. Bioactive components in rosehip flour extract: GC–MS analysis

Peak ID	Name	FID		
		RT (min)	Area	Area %
1	Palmitic acid (C16:0)	23.303	231654	4.97
2	Linoleic acid (C18:2w6)	28.041	2368444	50.83
3	alpha-Linolenic acid (C18:3w3)	28.161	903240	19.39
4	Oleic acid (C18:1w9)	28.255	1060018	22.75
5	Stearic acid (C18:0)	29.004	96011	2.06

Heavy Metal Content

The concentrations of heavy metals and mineral substances in both the powder and the seeds of the plant were determined. The findings obtained are presented in Table 4, expressed in milligrams per kilogram (mg/kg).

Table 4. Concentrations of heavy metals in rosehips (mg/kg)

Metals	Rosehip powder	Seed	ICP-MS LOD concentrations (µg/L)
Li	<LOD*	<LOD	0.179
B	<LOD	<LOD	2.180
V	0.064	0.143	3.592
Se	<LOD	<LOD	0.380
Mo	<LOD	<LOD	1.096
Na	<LOD	<LOD	2.044
Mg	1639.1	1929.5	1.3331
Al	21.5	62.7	0.024
K	9134.1	1006.4	0.014
Ca	622.8	747.3	0.334
Tl	<LOD	<LOD	0.001
Pb	<LOD	10.5	0.003
Bi	<LOD	<LOD	0.086
Si	92.4	317.7	0.204
As	<LOD	0.895	0.004
Be	<LOD	<LOD	0.041
Ti	2.4	2.4	0.047
Mn	36.9	77.5	0.014
Fe	45.5	97.8	0.781
Hg	<LOD	0.010	0.025
Co	<LOD	<LOD	0.003
Ni	0.590	3.753	0.018
Cu	10.5	4.5	0.842
Zn	5.7	25.8	4.610
Ga	3.1	2.5	0.019
Sr	22.2	92.8	0.004
Ag	<LOD	<LOD	0.009
Cd	<LOD	<LOD	0.008
In	<LOD	<LOD	0.001
Cr	<LOD	6.7	0.243
Ba	12.3	10.4	0.007
Sb	<LOD	<LOD	0.004

*LOD: Limit of detection

CONCLUSION

In this research, the characteristics of bioactive components in rosehip fruit powder were investigated through supercritical carbon dioxide extraction, including the analysis of total phenolic content, antioxidant activity (DPPH radical scavenging activity), and total flavonoid content of ground rosehip wild fruit along with its seeds. Additionally, the heavy metal content of the extract was analyzed using gas chromatography-mass spectrometry. The findings were compared with data from similar studies.

According to the research results, the characteristics of bioactive components in rosehip powder were generally found to be higher than those reported by various domestic and foreign researchers. However, differences observed in some parameters were attributed to the extraction method used.. This research demonstrates that supercritical carbon dioxide extraction on rosehip fruit powder is an effective method for revealing and characterizing the bioactive components of the fruit.

The findings of this study indicate that rosehip fruit is a significant source of potential health benefits and functional properties. The wild-grown fruit, which is economically undervalued, has been shown to contain important food components that can positively impact consumer health. These findings support the idea that rosehip fruit could be considered a functional food, and its consumption could be increased by incorporating it into various new food products. It is believed that this fruit, which can be grown in almost every region of the country, could contribute significantly to the food industry and the national economy. Therefore, further research is needed to expand the areas of utilization for rosehip fruit and to increase its consumption. Such research could highlight the health benefits of the fruit and lead to the development of new products. In conclusion, given the importance and potential of rosehip fruit, it is essential to encourage its consumption and explore new evaluation methods. This way, this fruit with positive effects on human health could reach a wider audience.

In the study, the content of elements present in different parts of the rosehip plant was analyzed. Element and heavy metal analyses in rosehip powder samples are important for identifying potential harmful substances in this plant and taking precautions for food safety. Such analyses are a crucial tool for protecting consumer health and determining the level of environmental pollution in foods.

Conflict of Interest Statement

The authors declare no conflict of interest.

Acknowledgments

We would like to thank AltraFlora Natural Extracts Inc. (Denizli, Türkiye) for their cooperation.

REFERENCES

[1] Duman, T. (2014). Kuşburnu (*Rosa canina*) nektarında toplam fenolik madde ve suda çözünen vitaminlerin ısıl parçalanma kinetiği. Yüksek Lisans Tezi. Pamukkale Üniversitesi, Fen Bilimleri Enstitüsü, Denizli.

[2] Davis, P.H., Cullen J., Coode M.J.E. (1965). Flora of Turkey and the East Aegean islands. University Press.

[3] Okcu, Z., Kerse, S., Yavuz, Y. (2017). Kuşburnu meyvesinin değişik ürünlere işlenirken besinsel kalitesindeki değişim. *Bahçe*, 46(special issue1), 89 - 96.

[4] Sapkota, B., Devkota, H.P., Poudel, A., Poudel, P., Thapa, R. (2023). Rosa spp (*Rosa canina* L., R. *macrophylla* Lindl., R. *moschata* Herrm., R. *multiflora* Thunb.). In Himalayan Fruits and Berries (pp. 371-381). *Academic Press*.

[5] Barros, L., Carvalho, A.M., Morais, J. S., Ferreira, I. C. (2010). Strawberry-tree, blackthorn and rose fruits: Detailed characterisation in nutrients and phytochemicals with antioxidant properties. *Food Chemistry*, 120(1), 247-254.

[6] Jäger, A.K., Eldeen, I.M.S., Van Staden, J. (2007). COX-1 and-2 activity of rose hip. *Phytotherapy Research*, 21(12), 1251-1252.

[7] Tumbas, V.T., Čanadanović-Brunet, J.M., Četojević-Simin, D.D., Ćetković, G.S., Đilas, S.M., Gille, L. (2012). Effect of rosehip (*Rosa canina* L.) phytochemicals on stable free radicals and human cancer cells. *Journal of the Science of Food and Agriculture*, 92(6), 1273-1281.

[8] Chrubasik, C., Roufogalis, B.D., Müller-Ladner, U., Chrubasik, S. (2008). A systematic review on the *Rosa canina* effect and efficacy profiles. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 22(6), 725-733.

[9] Tipi, E. (1996). Kuşburnu fidan üretim teknikleri ve üretim hedefleri. Kuşburnu Sempozyumu, 5-6.

[10] Kadakal, C., Nergiz, C. (1999). Kuşburnu çekirdeğinin mineral madde, yağ asidi içeriği ve radyasyon düzeyi. *Gıda Bilimi ve Teknolojisi*, 4, 59-64.

[11] Kadakal, Ç., Nas, S., Artık, N. (2002). Kuşburnu (*Rosa canina* L.) meyve ve çekirdeğinin bileşimi ve insan beslenmesi açısından önemi. *Dünya Gıda*, 7, 111-117.

[12] 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.

[13] Kadakal, Ç., Gürsoy, O., Nergiz, C. (1999). Gümüşhane yöresinde doğal olarak yetişen kuşburnu (*Rosa canina* L.) bitkisinin meyve ve çekirdeğinin bazı fiziksel ve kimyasal özellikleri. *Gıda Bilimi ve Teknolojisi Dergisi*, 4, 34-41.

[14] Rozzi, N.L., Phippen, W., Simon, J.E., Singh, R.K. (2002). Supercritical fluid extraction of essential oil components from lemon-scented botanicals. *LWT-Food Science and Technology*, 35(4), 319-324.

[15] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D.H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19(6-7), 669-675.

[16] Singleton, V.L., Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158.

[17] Zhishen, J., Mengcheng, T., Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555-559.

[18] Su, L., Yin, J.J., Charles, D., Zhou, K., Moore, J., Yu, L.L. (2007). Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food Chemistry*, 100(3), 990-997.

[19] Montazeri, N., Baher, E., Mirzajani, F., Barami, Z., Yousefian, S. (2011). Phytochemical contents and biological activities of *Rosa canina* fruit from Iran. *Journal of Medicinal Plants Research*, 5(18), 4584-4589.

[20] Nađpal, J.D., Lesjak, M.M., Šibul, F.S., Anačkov, G.T., Četojević-Simin, D.D., Mimica-Dukić, N. M., Beara, I. N. (2016). Comparative study of biological activities and phytochemical composition of two rose hips and their preserves: *Rosa canina* L. and *Rosa arvensis* Huds. *Food Chemistry*, 192, 907-914.

[21] Agourram, A., Ghirardello, D., Rantsiou, K., Zeppa, G., Belviso, S., Romane, A., Giordano, M. (2013). Phenolic content, antioxidant potential, and antimicrobial activities of fruit and vegetable by-product extracts. International *Journal of Food Properties*, 16(5), 1092-1104.

[22] Macit, M. (2018). *Rosa canina* L. ve *Rosa pimpinellifolia* L. Köklerindeki fenolik bileşiklerin miktarı ve bioyararlılığının tespiti. İstanbul Üniversitesi. Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı, Yüksek Lisans Tezi, İstanbul, 92s.

[23] Demir, N., Yıldız, O., Alpaslan, M., Hayaloğlu, A.A., (2014). Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (*Rosa canina* L.) fruits in Turkey, *Food Science and Technology*, 57(1), 126-133.

[24] Murathan, Z.T., Zarifikhosroshahi, M., Kafkas, E., Sevindik, E. (2016). Characterization of bioactive compounds in rose hip species from East Anatolia region of Turkey. *Journal of Food Science*, 28(2):314-325.

[25] Fattahi, S., Jamei, R., Hosseini Sarghein, S., (2012), Antioxidant and antiradical activities of *rosa canina* and *rosa pimpinellifolia* fruits from west

Azerbaijan, *Journal of Plant Physiology*, 4, 523-529.

[26] Gao, X., Björk, L., Trajkovski, V., Uggla, M. (2000). Evaluation of antioxidant activities of Rosehip ethanol extracts in different test systems. *Journal of Science of Food and Agriculture*, 80, 2021-2027.

[27] Marevci, M.K., Žitek, T., Postružnik, V., Knez, Ž. (2022). Extraction of phenolic compounds from white and red grape skin and rosehip fruit. *Journal of Hygienic Engineering & Design*, 39.