Journal of Anatolian Environmental and Animal Sciences
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
DOI: https://doi.org/10.35229/jaes.1567422

JAES
Year: 9, No: 4, 2024 (711-720)
AÇEH
Yıl: 9, Sayı: 4, 2024 (711-720)

ARAŞTIRMA MAKALESİ RESEARCH PAPER

Effect of Different Storage Conditions and Infusion Times on Antioxidant and Antimicrobial Activites and Total Phenolic Content of Rosehip Fruits [*]

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Received: *15.10.2024* ***Accepted:*** *09.12.2024* ***Published:*** *31.12.2024*

How to cite: **Darak, M., Kılınç, K., Akar, Z., Özad Düzgün, A., Acet, T., Ural, M.N., C. Baltacı & Akmeşe, O. (2024).** Effect of Different Storage Conditions and Infusion Times on Antioxidant and Antimicrobial Activites and Total Phenolic Content of Rosehip Fruits. *J. Anatolian Env. and Anim. Sciences*, ***9***(4), 711-720. https://doi.org/10.35229/jaes.1567422

Atıf yapmak için: **Darak, M., Kılınç, K., Akar, Z., Özad Düzgün, A., Acet, T., Ural, M.N., C. Baltacı & Akmeşe, O. (2024).** Farklı Saklama Koşulları ve İnfüzyon Sürelerinin Kuşburnu Meyvelerinin Antioksidan ve Antimikrobiyal Aktiviteleri ile Toplam Fenolik İçeriği Üzerine Etkisi. *Anadolu Çev. ve Hay. Dergisi*, ***9***(4), 711-720. https://doi.org/10.35229/jaes.1567422

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Abstract: *Rosa canina L.* (Rosehip) is a medicinal plant with strong antioxidant properties. The content of rosehip and accordingly its antioxidant capability may vary according to the way the fruits are used, storage, infusion, freezing and drying methods. In study, it was aimed to determine the antioxidant, antimicrobial activities in rosehip infusion products and extracts under these conditions. Fresh, frozen, dried and +4^{0}C stored fruits were infused at 90 degrees and infusion groups were formed. During infusion, samples were taken at 30, 60, 90, 120, 300 and 360th minutes and antioxidant activity (DPPH, FRAP and ABTS methods), total phenolic and total flavonoid contents (TPC and TFC), and antimicrobial activity were analyzed. In addition, phenolic compound content was investigated in 3 different extracts by HPLC method. Dried rosehip (DR) infusing samples were found to have the highest antioxidant activity, TPC and TFC compared to all groups (between $p< 0.005$ and $p=0.05$).). Significant decreases were detected in the parameters according to storage and freezing times. A total of 8 phenolic compounds, the highest concentration of which was epicatechin, were detected in aqueous and ethanolic extracts of dried and stored at 4^{0}C rosehip (DR+4^{0}C) and dried rosehip (DR) fruits. For antimicrobial analysis, Total 7 different Gram (+) and (Gram (-) bacterial strains, were used. The highest activity against *E. coli* was observed in ethanolic DK extract (dried and frozen at -20 ^{0}C). While antimicrobial activity was observed in the extracts, no activity was observed in the infusion products. It was determined that antioxidant activity increased during 360-minute infusion time, although it varied according to storage conditions.

Keywords: Antimicrobial, antioxidant, infusion, storage, rosehip.

Farklı Saklama Koşulları ve İnfüzyon Sürelerinin Kuşburnu Meyvelerinin Antioksidan ve Antimikrobiyal Aktiviteleri ile Toplam Fenolik İçeriği Üzerine Etkisi

Öz: Rosa canina L. (Kuşburnu) güçlü antioksidan özelliklere sahip tıbbi bir bitkidir. Kuşburnunun içeriği ve buna bağlı olarak antioksidan özelliği meyvelerin kullanım şekline, depolama, infüzyon, dondurma ve kurutma yöntemlerine göre değişebilmektedir. Çalışmamızda, sözkonusu koşullar altında kuşburnu infüzyon ürünleri ve ekstraktlarında antioksidan, antimikrobiyal aktivitelerdeki değişimlerin belirlenmesi amaçlandı. Taze, dondurulmuş, kurutulmuş ve +4^{0}C'de depolanmış meyveler 90 ^{0}C'de demlenmesiyle demleme grupları oluşturuldu. Demleme sırasında 30, 60, 90 ve 120. 300 ve 360. dakikalarda örnekler alınarak ve antioksidan aktivite (DPPH, FRAP ve ABTS yöntemleri ile), toplam fenolik (TPC), toplam flavonoid içerikleri (TFC) ve antimikrobiyal aktiviteleri analiz edildi. Ayrıca 3 farklı ekstraktta HPLC yöntemi ile fenolik bileşik içeriği araştırıldı. Kurutulmuş kuşburnu (DR) demleme örneklerinin tüm gruplara kıyasla en yüksek antioksidan aktiviteye, TPC ve TFC değerlerine

[*] *This study is based on the master's thesis (Darak, 2022) presented at Gumushane University , Graduate Education Institute*

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sahip olduğu bulundu (p <0.001 – p=0.05 arasında). Saklama ve dondurma sürelerine göre parametrelerde anlamlı azalmalar tespit edildi. Kurutulmuş ve 4^0C'de saklanmış kuşburnu (DR+4^0C) ve kurutulmuş kuşburnu (DR) meyvelerinin sulu ve etanolik ekstraktlarında en yüksek konsantrasyonu epikateşin olan toplam 8 fenolik bileşik tespit elde edildi. Antimikrobiyal analiz için toplam 7 farklı Gram (+) ve Gram (-) bakteriyel suş kullanıldı. En yüksek aktivite E. *c*oli'ye karşı etanolik (kurutulmuş ve – 20 0C'de dondurulmuş) DK ekstraktında görüldü. Ekstraktlarda antimikrobiyal aktivite gözlenirken, infüzyon ürünlerinde herhangi bir aktivite görülmedi. Saklama koşullarına göre değişmekle beraber antioksidan aktivite değerlerinin 360 dakika demleme süresi boyunca arttığı tespit edildi.

Anahtar kelimeler: Antimikrobiyal, antioksidan, demleme, kuşburnu, saklama.

INTRODUCTION

Plants produce various organic compounds, called secondary metabolites, for adaptation to various conditions and defence purposes (Tiring et al., 2021). Medicinal plants are a group of plants used in traditional and modern treatment methods that produce bioactive seconder metabolites with extremely different effects such as anticancer, antidiabetic, antihypertensive, anti-inflammatory, antimicrobial, anticytotoxicity, wound healing, DNA repair (Faydaoğlu & Sürücüoğlu, 2011; Göktaş & Gıdık, 2019; Riaz et al., 2023;Turan et al, 2019). It is stated that the rich phenolic and vitamin content of medicinal plants play a protective role due to their antioxidant properties against oxidative effects, which have been shown to play a role in the development of diseases. (Kılınç et al., 2020; Riaz et al., 2023).

Rosa canina (Rosehip), a member of the Rosaceae family, has more than 100 species and has a wide distribution in Western Asia, Middle East, Northern and Central Europe and North America (Oz et al., 2019; Roman et al., 2013). The fruits of the plant, ripens and blushes in early autumn and turn orange, pink or reddish colors. The fruits are collected in September-October months and are widely used in the production of fruit juice, jam and marmalade, especially tea. In addition to its consumption as a food source, rosehips are traditionally used in the treatment of diseases such as colds, infections, inflammation, haemorrhoids, chronic pain and arthritis (Kilinc et al., 2020; Selahvarzian et al., 2018;Turan et al., 2018). Bioactive compound analyses of rosehip fruits showed that they have rich compound content such as vitamins, organic acids, flavonoids, tannins, fatty acids, pectins and caretenoids. It is also one of the fruit varieties with the highest vitamin C content (Negrean et al., 2024; Nadpal et al., 2016; Roman et al., 2013). The high phenolic and vitamin content of fruits reveals its strong antioxidant properties.

Although the rosehip plant is found in almost every region in Türkiye, it is mostly distributed in the Central and Eastern Black Sea, Central Anatolia and Eastern Anatolia Regions (Kocaman & Sormaz, 2023; Oz et al., 2018). Tea consumption is quite common throughout the world and there are many different types of tea preferred according to cultures. Among these types, fruit teas are a group that is widely consumed especially for their health benefits (Zohora & Arefin, 2022). Rosehip tea is one of the herbal teas that has commercial value in Türkiye and worldwide, is widely consumed and has important health effects (Akar, et al., 2021; Choi, 2009). It is mostly sold in dried form, tea bags, dried whole fruit, seedless fruit, hibiscus or mixed with other herbal products with antioxidant content. Consumption increases especially in winter. It is also available in the form of fruit juice. It is collected by the local people in September and October, dried or frozen and stored and used.

Rosehip fruits can be easily collected from plants in the form of bushes of several metres and can be used directly as tea, fresh or dried, without further processing. Rosehip tea is widely consumed in Anatolia, especially in rural regions, by boiling in teapots for a long time (Kocaman & Sormaz, 2023). This is important in terms of easy and effective utilisation of antioxidant and vitamins properties of fruits.

Freezing, drying or a combination of both are common storage methods for fruit and vegetables. In the food industry, the stages of the products' journey from raw materials to the consumer can result in alterations to the chemical and bioactive structures of the products (Toydemir et al., 2021). Consuming plant parts dry or wet, drying and storage conditions (temperature and time), and methods used during processing can affect the antioxidant content and capacity of the plant. Therfore, in order for antioxidant and other protective-healing properties to effective, attention should be paid to collection, drying, storage and preparation methods (Safarov, 2020; Giannakourou & Taoukis, 2021).

Rosehip fruits are a widely consumed plant in Türkiye. Drying and preserving of rosehip fruits is the most widely used traditional method (Gruszecki et al., 2024). In this study, the objective was to ascertain the changes in the antioxidant and antimicrobial properties of dried and infused samples with some extracts of rosehip fruits depending on storage conditions and infusion time. Additionally, the aim was to identify the optimal conditions for these properties.

MATERIAL AND METHOD

Collection and grouping of samples: Rosehip fruits were harvested at the end of September, which is the

ripening period in 2021, from the same plant bush group in the vicinity of Gümüşkaya village on Gümüşhane - Şiran road, at an altitude of approximately 1300 metres. The fruits were delivered to the laboratory in a sealed bag. In the study, infusion products and extracts were coded and grouped as follows:

(1)FR: Fresh rosehip (freshly picked and untreated); **(2)DR:** Dried rosehip (dried in an oven at in 36^{0}C); **(3)FF:** Frozen freshly rosehip (fruits were frozen at -20^{0}C for 3 months; **(4)DR+4^{0}C;** Dried rosehip stored at +4^{0}C for 3 months; **(5)FFDR:** sample of rosehips frozen at -20^{0}C and then thawed and dried after 3 months; **(6)DRIF:** Sample of infused FF sample stored at -20^{0}C for 3 months; (**7)FRIF**: Sample of infused FR stored at -20^{0}C for 3 months; **FFDR-EtE:** ethanolic extract of Freezing fresh rosehip fruits at -20^{0}C, thawing and drying after 3 months; **DR-WE and DR-EtE:** Aqueous and ethanolic extracts of DR samples, **DR+4^{0}C EtE**: Ethanolic extract of dried rosehip kept at +4^{0}C for 3 months (Figure 1).

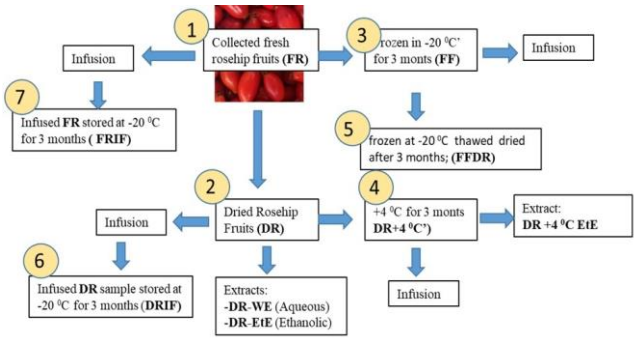

Figure 1. Rosehip infusion and extract groups used in the study.

***Extraction**:* Some of the fresh rosehip (FR) samples were dried in an oven (Memmert, Germany) at 36 ^{0}C for 48h (DR). DR fruits were pulverised with the help of a blender for aqueous and ethanolic extraction by removing the seeds and 5 grams of these samples were weighed and added to a volume of 50 mL with pure water or ethanol according to the method. After vortexing, the samples were incubated in a shaker incubator (Shel Lab, UK) at 150 rpm at 45 °C for 24 hours in the dark. The samples were then filtered through filter paper and transferred to a balloon jar. A rotary evaporator (Heildolph, Germany) was used to evaporate the solvent in the flask. The residues were dissolved with a small amount of distilled water or ethanol. Some of the extracts obtained were stored at +4 °C and some were stored at -20 °C for further studies

Infusion: At different times, samples coded as FR, DR, FF, DR+4 ^{0}C and FFDR were weighed on a precision balance (KERN, Switzerland) of 25 g each (commercially sold bag tea products are usually between 2.5 -5 grams in a 150 -200 mL glass volume). The volume was completed to 500 mL with drinking water from a commercially available company and transferred to a teapot. The teapots were placed on a heater (IKA, Germany) fixed at 90 ^{0}C with the lid closed. At 30, 60, 90 and 120 minutes and at 5 and 6 hours, 10 mL each samples was taken from the teapot, passed through coarse filter paper and placed in a 15 mL conic santrifuge tube. For all groups, infusion process was carried out in the same teapot at separate times. Drinking water of the same company was used in all samples during period. FR and DR infusion samples were stored at -20 ^{0}C for 3 months to be used in the same analyses (FRIF – DRIF, products respectively). All infusion samples and standards were run in 3 replicates except HPLC analysis.

Determination of total phenolic content (TPC): This assay was adapted from the method of Acet & Ozcan (2018) and Beara et al. (2009) adapted to 96-well microplate from Singleton et al., (1999). Gallic acid prepared in 6 different concentrations was used as standard. 31 µL of infused samples and gallic acid standards were pipetted into a 96-well plate. Folin-Ciocalteu reagent (1:10, v/v) was added to them and it was waited 10 minutes for reaction in the dark. Finally of 1% sodium carbonate (Na_2CO_3) solution was pipetted to all well. Total volume of pleyt well was 250 µL. After incubation for 2 h at room temperature, reads were taken at 760 nm in a microplate reader (Thermo Sci. MultiScan USA). All samples and standards were run in 3 replicates. Results were given gallic acide equivalent (µg/mL GAE) (Table 2).

Determination of total flavonoid content (TFC): TFC analysis was performed by revising the aluminium chloride ($AlCl_3$) colorimetric method of Beara et al. adapted to 96-well microplate. Quercetin prepared in 6 different concentrations was used as standard for calibration graph. 30 µL of the infused samples and standards were pipetted on the microplate. Then methanol, $AlCl_3$ (10%), CH_3COOK (1 mol/L) and distilled water was added respectively. Final volume was 250 µL. After pipetting, the microplate was kept at room temperature in a dark environment for 40 minutes. and then read on a microplate reader at 415 nm wavelength. Results were given as quercetin equivalent (µg/mL QAE) (Table 3).

Determination of antioxidant activities: In analyses, each samples and standart were run in 3 replicates.

DPPH radical scavenging assay: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical capture method was carried out by adapting the method used by Soler-Rivas et al. (2000) to 96-well microplate. 10 µL infused samples and gallic acid standards prepared at 6 different concentrations were pipetted on to a 96-well plate. DPPH solution (90 µmol/L) and than methanol were added. Final volume was 300 µL. Reaction was kept in the dark for 1 hour under room conditions. At the end of waiting period, reading were taken on a microplate reader at 515 nm wavelength. Results were

given as µg/mL gallic acid equivalent (µg/mL GAE) (Table 4).

Ferric reducing antioxidant power (FRAP) assay: The method was applied by adapting the ferric reducing antioxidant potential (FRAP) method of Bezie and Strain, (1996) to 96-well microplate. Trolox was used for standard calibration in the experiment. 25 µL of infused samples and trolox standards prepared at 6 different concentrations were pipetted into a 96-well microplate. Than FRAP reagent [300 mM acetate buffer + 10 mM 2,4,6-Tri(pyridy)-S-triazine (TPTZ) solution + 20 mM iron III chloride ($FeCl_3$) solution (10:1:1)] was added. Total volume was 300 µL in each well. Microplate was incubated for 30 minutes at room temperature and measured at 593 nm wavelength on a microplate reader. Results were given as µg/mL TEAC (Trolox Equivalent Antioxidant Capacity) (Table 5).

ABTS scavenging activity assay: This method was modified from the method adapted by Acet and Ozcan, (2018), Silva et al., (2007) for 96-well microplates. Briefly, 80 µL of the infusion products and different concentrations of trolox standards were pipetted into the microplate wells. ABTS (2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) radical solution (7 mM ABTS + 2.45 mM potassium persulfate) with an optical density of 0.7 was added. Final volum was 300 µL. The plate was incubated in the dark at 30°C for 5-6 minutes. It was read on a microplate reader at 734 nm wavelength. Results were given as µg/mL TAEC (Trolox Equivalent Antioxidant Capacity) (Table 6).

Phenolic compounds analysis by HPLC: Phenolic compound analysis of rosehip samples was carried out in Gumushane University Central Laboratory Research and Application Centre by using diode array detector (DAD) in high performance liquid chromatography (HPLC) device (Agilent Infinity ABD 1200 series) acording to the method of Paje et al., (2022). Macherey-Nagel brand, EC 250x4.6 nucleosyl 100-5, 5 µm C18 HD column was used in the analysis. The column oven temperature was 27 ºC. The mobile phase components were set as ultrapure water (phase A), 100% acetonnitrile (phase B), 3% acetic acid/ultra pure water (phase C) and 3% acetic acid + 25% acetonnitrile + ultrapure water (phase D) at a flow rate of 1 mL/min. 210-360 nm wavelength scanning was performed with DAD. Gallic acid, protocatechuic acid, protocatechuic aldehyde, catechin, 4-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, epicatechin, vanillin, p-coumaric acid, benzoic acid, ferulic acid, rutin, rosmarinic acid, coumarin, t-cinnamic acid, quarcetin and kaempherol were used as standart respectively (Figure 2). Their analytical method validation parameters are given in Table 1.

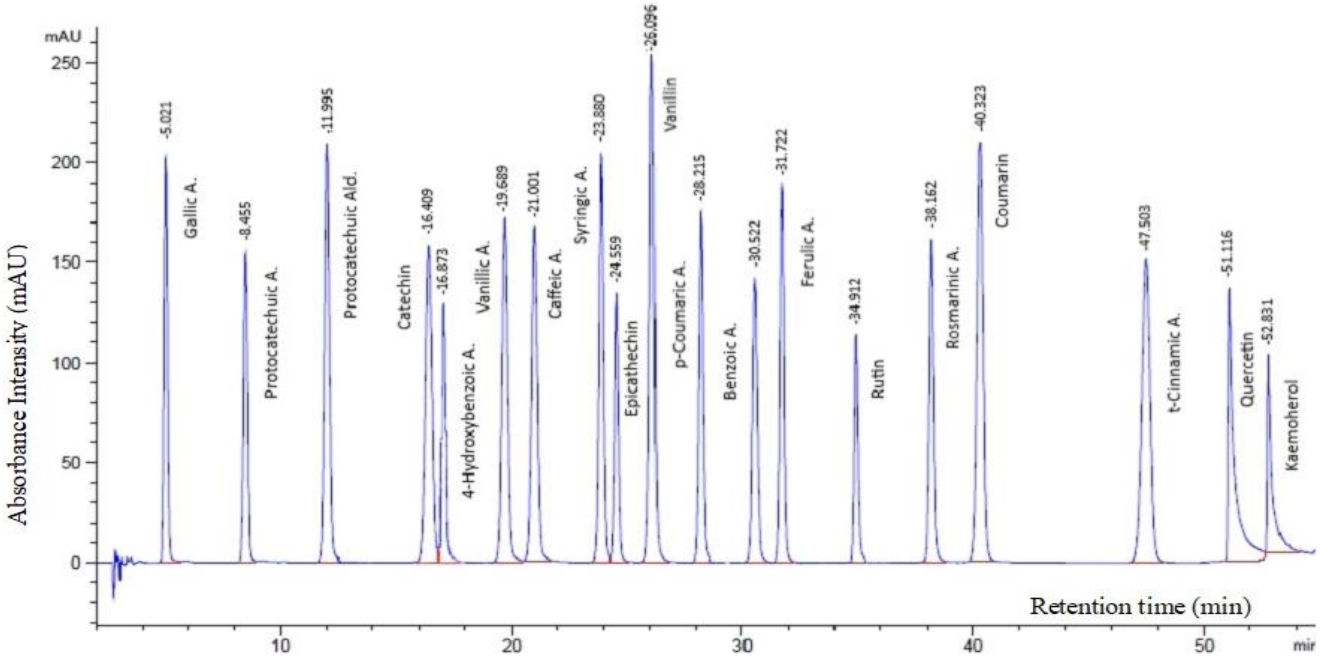

Figure 2. HPLC standard chromatogram and standards used.

Antimicrobial activity: Agar disk diffusion method was applied for antimicrobial activity analysis. *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *S. pyogenes* ATCC 19615 as Gram (+) bacteria strains; *E. coli* ATCC 25922, *P. aeruginosa* ATCC43288, *Proteus vulgaris* ATCC 13315, *Yersinia pseudotuberculosis* ATCC 911 as Gram (-) bacteria starins were used. The petri dishes were covered with agar medium and 6 mm diameter wells were made on the medium with a sterile puncher. 50 µL volumes of infused samples (50 mg/mL), FFDR ethanolic extract (51.1 mg/mL), DR ethanolic extract (DR-EtE, 87.3 mg/mL) and DR aqueous extract (DR-WE, 385 mg/mL) were pipetted into wells. Ampicillin (50 mg/mL) was used as a control. Dishes were kept at room temperature for 2

hours and then incubated in incubator at 37 ^{0}C overnight. The zones were measured and evaluated with the help of a caliper (Akay & Sefaoğlu, 2021). Zones over 6 mm in diameter were considered significant.

Statistics: Except for HPLC analyses, the results of the experiment performed in 3 replicates. Results were calculated by One-Way Anova test using statistic programme (IBM SPSS ver. 26). Tukey's test was used for comparisons between groups. Results are given as mean values. Statistically p <0.05 was accepted statistically significant.

RESULTS

TPC and TFC: TPC and TFC results are shown in Table 2 and Table 3 respectively.

Table 1. Analytical method validation parameters of phenolic compounds analyzed in HPLC

No	Analytes	RT	Correlation	r^2	RSD_R%	RSD_r%	Linearity Range (mg/L)	*LOD/LOQ* (mg/L)	Recovery(%)
					Interday	Intraday			
1	Gallic A.	5.021	*y*=171.892x -16.386	0.995	2.18	1.52	5-100	1.27/4.22	100.0
2	Protocatechuic A.	8.455	*y*=- 34.326x -3.408	0.999	1.30	0.63	5-100	0.80/2.65	99.8
3	Protocatechuic Ald.	11.995	*y*=- 164.513x +20.025	0.999	2.42	1.48	5-100	1.02/3.39	99.8
4	Catechin	16.409	*y*=5.359x -63.413	0.999	1.24	0.81	5-100	1.68/5.48	100.1
5	4-Hydroxybenzoic A.	16.873	*y*=- 36.111x -8.048	0.999	1.30	1.02	5-100	2.03/6.78	99.6
6	Vanillic A.	19.689	*y*=- 39.531x +9.3444	0.998	2.11	1.09	5-100	1.31/4.36	99.7
7	Caffeic A.	21.001	*y*=-78.796x -11.629	0.971	2.42	0.93	5-100	1.25/4.18	99.6
8	Syringic A.	23.880	*y*=- 71.521x+6.906	0.998	1.18	0.63	5-100	0.75/2.50	99.7
9	Epicatechin	24.559	*y*=-17.582x +5.666	0.999	2.11	1.08	5-100	0.50/1.65	100.2
10	Vanillin	26.096	*y*=- 95.730x +4.534	0.998	2.18	1.09	5-100	0.41/1.37	99.7
11	P Coumaric A.	28.215	*y*= 119.591x -12.267	0.999	2.40	1.16	5-100	0.91/3.04	99.7
12	Benzoic A.	30.522	*y*=-9.716x-2.535	0.999	2.40	0.51	5-100	1.41/4.71	100.1
13	Ferulic A.	31.722	*y*=-68.387x -32.358	0.997	2.18	1.22	5-100	0.77/2.56	99.6
14	Rutin	34.912	*y*=- 16.562x -2.353	0.998	1.92	0.76	5-100	1.26/4.19	100.2
15	Rosmarinic A.	38.162	*y*=- 40.241x -1.551	0.996	1.47	0.62	5-100	0.32/1.07	100.1
16	Coumarin	40.323	*y*=-101.687x +17.691	0.998	1.47	0.73	5-100	0.47/1.55	99.6
17	t-cinnamic A.	47.503	*y*=- 194.844x -25.077	0.998	2.12	1.48	5-100	1.24/4.15	99.7
18	Quercetin	21.116	*y*=- 27.251x -1.527	0.999	1.94	0.97	5-100	0.71/2.33	100.3
19	Kaempherol	52.831	*y*= 15.443x-0.862	0.998	2.40	0.57	5-100	0.59/1.97	100.0

A: Acid, **Ald:**Aldehyde, **RT**: Retention Time, **LOD:** Limit of detection, **LOQ**: Limit of Quantitation

Table 2. Total phenolic content (TPC) of rosehip groups according to infusion times

Time	Phenolic substance quantities (µg/mL GAE)						
	FR	DR	FF	DR+4 ^{0}C	FFDR	DRIF	FRIF
30 min	165.8$^{a.c.d.e.f}$	336.8$^{(*)}$	116.9$^{a.b.e.f.g}$	101.6$^{a.b.e.f.g}$	181.6$^{a.c.d.e.f}$	64.0$^{(*)}$	42.8$^{(*)}$
60 min	243.1$^{a.c.d.e.f}$	464.7$^{(*)}$	157.1$^{a.b.e.f.g}$	144.3$^{a.b.e.f.g}$	292.9^{a,c,d,e,f}	80.7$^{(*)}$	45.8$^{(*)}$
90 min	274.8$^{(*)}$	568.5$^{(*)}$	220.7$^{(*)}$	182.5$^{(*)}$	325.4$^{(*)}$	85.3$^{(*)}$	67.1$^{(*)}$
120 min	321.6$^{a.c.d.e.f}$	629.3$^{(*)}$	236.2$^{a.b.e.f.g}$	238.3$^{a.b.e.f.g}$	353.7$^{a.c.d.e.f}$	127.6$^{(*)}$	74.7$^{(*)}$
300 min	447.4$^{a.d.e.f}$	891.9$^{(*)}$	300.3$^{(*)}$	479.5$^{a.d.e.f}$	434.6$^{a,d.e.f}$	185.1$^{(*)}$	75.7$^{(*)}$
360 min	447.6$^{a.c.d.e.f}$	914.5$^{(*)}$	352.2$^{(*)}$	599.2 $^{(*)}$	470$^{a.c.d.e.f}$	216.1$^{(*)}$	84.7$^{(*)}$

(*) : Significant difference according to all groups; **a**: According to **DR**; **b:** According to **FR; c:** According to **DR+4 ^{0}C; d**:According to **FF; e:** According to **DRIF; f:** According to **FRIF**; **g:** According to **FFDR**. p<0.05 was considered statistically significant.

Table 3. Total flavonoid content (TFC) of rosehip groups according to infusion times

Time	Flavonoid substance quantities (µg/mL QE)						
	FR	DR	FF	DR+4 ^{0}C	FFDR	DRIF	FRIF
30 min	740$^{(*)}$	2010$^{(*)}$	437.7$^{(*)}$	1512.5$^{a.b.d.e.f}$	1379.1$^{a.b.d.e.f}$	140.3$^{a.b.c.d.g}$	116.8$^{a.b.c.d.g}$
60 min	935$^{(*)}$	3045$^{(*)}$	490$^{(*)}$	1590.8$^{a.b.d.f.e}$	1515$^{a.b.d.e.f}$	145.7$^{a.b.c.d.g}$	122.6 $^{a.b.c.d.g}$
90 min	1420$^{(*)}$	4940$^{(*)}$	509.9$^{(*)}$	1722.7$^{a.b.d.e.f}$	1620 $^{a.b.d.e.f}$	195.5$^{a.b.c.d}$	131.9 $^{a.b.c.d}$
120 min	1435$^{a.c.d.e.f}$	5210$^{(*)}$	637.2$^{(*)}$	1751.6$^{a.b.d.e.f}$	1810.5$^{a.b.d.e.f}$	242.9$^{a.b.c.d.g}$	157.7$^{a.b.c.d.g}$
300 min	1957.5$^{a.d.e.f}$	5470$^{(*)}$	663.3$^{(*)}$	1988.3$^{a.d.e.f}$	1862.7$^{a.d.e.f}$	255.3$^{a.b.c.d.g}$	194.2$^{a.b.c.d.g}$
360 min	2735$^{(*)}$	6730$^{(*)}$	709.4$^{(*)}$	2070$^{a.b.d.e.f}$	1943.3$^{a.b.d.e.f}$	265.1$^{a.b.c.d.g}$	214.1$^{a.b.c.d.g}$

(*) : Significant difference according to all groups; **a**: According to **DR**; **b:** According to **FR; c:** According to **DR+4 ^{0}C; d**:According to **FF; e:** According to **DRIF; f:** According to **FRIF**; **g:** According to **FFDR**. p<0.05 was considered statistically significant.

It was determined that all of the infusion samples of the rosehip groups showed an increase in both TPC (Table 2) and TFC (Table 3) from the 30th minutes to the end of the 360th minute. Among the groups, the dried rosehip group (DR) had higher values than all other groups at all time points in both TPC and TFC groups (p<0.001). A comparison of FF and FFDR revealed that the latter exhibited significantly higher TPC and TFC values at all infusion times. On the other hand, there was no significant difference between DR+4^{0}C and FFDR in terms of TFC during the infusion periods. The dried rosehip infusion samples (DR, DR+4 ^{0}C and FFDR) had higher flavonoid content than the frozen rosehip samples (FF) during the whole infusion period. FRIF and DRIF groups had significantly lower values in terms of both TPC and TFC compared to the other groups.

Results of antioxidant activity: It was observed that DPPH values continued to increase in all groups

depending on the infusion time (Table 4). At 60th minutes, there was a strong increase in FF, DR, FF, and DR+4 ^{0}C samples. After the 300th minute, there was a slowdown in activity increases in these groups. The samples of FF demonstrated consistently higher activity than those of FFDR samples. It was observed that the DR infusion samples exhibited the highest DPPH values in comparison to the other samples from the 60th minute onwards. FR samples had the second highest values after DR from 60th minutes starting.

Table 4. DPPH values of rosehip groups according to infusion times

Time	DPPH values (µg/mL GAE)						
	FR	**DR**	**FF**	**DR+4 ^{0}C**	**FFDR**	**DRIF**	**FRIF**
30 min	196.5$^{c.d.e.f.g}$	204.5$^{c.d.e.f.g}$	383.1$^{(*)}$	163.4$^{(*)}$	236.2$^{(*)}$	77.4$^{(*)}$	102.6$^{(*)}$
60 min	595.7$^{(*)}$	733.2$^{(*)}$	507.5$^{(*)}$	466.0$^{(*)}$	291.3$^{(*)}$	110.3$^{(*)}$	139.0$^{(*)}$
90 min	730.2$^{c.d.e.f.g}$	739$^{c.d.e.f.g}$	558.2$^{a.b.e.f.g}$	583.8$^{a.b.e.f.g}$	323.1$^{(*)}$	133.6$^{a.b.c.d.g}$	145.2$^{a.b.c.d.g}$
120 min	741.7$^{c.d.e.f.g}$	775$^{c.d.e.f.g}$	682.2$^{a.b.e.f.g}$	665.3$^{a.b.e.f.g}$	362.5$^{(*)}$	201.3$^{(*)}$	153.0$^{(*)}$
300 min	769.7$^{(*)}$	874.7$^{(*)}$	839.5$^{(*)}$	800.4$^{(*)}$	396.0$^{(*)}$	209.8 $^{a.b.c.d.g}$	224.6$^{a.b.c.d.g}$
360 min	788.2$^{(*)}$	906$^{(*)}$	870.8$^{a.b.e.f.g}$	862.0$^{a.b.e.f.g}$	419.9$^{(*)}$	309.4$^{(*)}$	248.4$^{(*)}$

(*) : Significant difference according to all groups; **a**: According to **DR**; **b:** According to **FR; c:** According to **DR+4 ^{0}C; d**:According to **FF; e:** According to **DRIF; f:** According to **FRIF**; **g:** According to **FFDR**. p<0.05 was considered statistically significant.

Table 5. FRAP values of rosehip groups according to infusion times

Time	FRAP values (µg/mL TEAC)						
	FR	**DR**	**FF**	**DR+4 ^{0}C**	**FFDR**	**DRIF**	**FRIF**
30 min	149.1$^{(*)}$	314.7 $^{(*)}$	96.2 $^{a.b.e.f.}$	78.4$^{a.b.e.f.g}$	119.1$^{a.b.e.f}$	45.7$^{a.b.c.d.g}$	24.1$^{a.b.c.d.g}$
60 min	239.2$^{(*)}$	504.2$^{(*)}$	114.5 $^{a.b.f.g}$	84.4$^{a.b.f.g}$	195.9$^{(*)}$	81.3$^{a.b.d.g}$	42.8$^{a.b.c.d.f}$
90 min	534,1$^{(*)}$	592.8$^{(*)}$	173.9$^{a.b.e.f.g}$	149.2$^{a.b.e.f.g}$	229.7$^{(*)}$	98.9$^{(*)}$	47.2$^{(*)}$
120 min	540.9$^{(*)}$	803.5$^{(*)}$	207.5$^{a.b.e.f.g}$	172.2$^{a.b.e.f.g}$	257.3$^{(*)}$	102.7$^{(*)}$	52.9$^{(*)}$
300 min	550.7$^{(*)}$	1197.2$^{(*)}$	275.7$^{a.b.c.e.f}$	359.0$^{a.b.e.f}$	305.8$^{a.b.e.f}$	173.4$^{(*)}$	84.9$^{(*)}$
360 min	606.9$^{(*)}$	1209.8$^{(*)}$	295.3$^{a.b.c.e.f}$	414.6$^{(*)}$	343$^{a.b.c.e.f}$	195$^{(*)}$	92.5$^{(*)}$

(*) : Significant difference according to all groups; **a**: According to **DR**; **b:** According to **FR; c:** According to **DR+4 ^{0}C; d**:According to **FF; e:** According to **DRIF; f:** According to **FRIF**; **g:** According to **FFDR**. p<0.05 was considered statistically significant.

In the FRAP test, it was observed that the DR infusion sample had the highest values at all times (p<0.001). As with the DPPH analysis, there was a notable increase in the samples of FR, DR, FF and DR+4^{0}C infusion between the 60th and 90th minutes. However, the increases were limited after 90 minutes. Once more, a notable increase was evident in the DR and DR+4 ^{0}C infusion samples between the 120th and 300th minutes (Table 4). FRIP and DRIP infusion groups showed low performance in both DPPH and FRAP analyses.

In antioxidant activity analyses of ABTS, it was determined that ABTS values of FR infusion samples were significantly higher than other infusion samples for all time periods (P<0.001). However, ABTS antioxidant capacities increased in all infusion groups from 30th minute to 360th min. (Table 6). After the 300th minutes, the rate of increase remained at a low level in all infusion products except for the FR group.

Phenolic content analysis: The analysis of phenolic content was conducted using high-performance liquid chromatography (HPLC). The samples employed were ethanolic and aqueous extracts of DR fruits, as well as an ethanolic extract of DR+4 ^{0}C fruits. 8 different phenolic compounds were detected (Table 7). Catechin was the compound with the highest concentration in ethanolic extracts of DR and DR+4 ^{0}C (661.42 and 428.53 µg/g extract, respectively).

Table 6. ABTS values of rosehip groups according to infusion times.

Time	ABTS (µg/mL TEAC)						
	FR	**DR**	**FF**	**DR+4^{0}C**	**FFDR**	**DRIF**	**FRIF**
30 min	529.5$^{a.c.d.f.g}$	1564.5 $^{(*)}$	577.7$^{(*)}$	418.8$^{(*)}$	333.2$^{(*)}$	528$^{a.c.d.f.g}$	286.9$^{(*)}$
60 min	812.9 a.c.e	1741.5 $^{(*)}$	754.9$^{a.c.e}$	567.8$^{a.b.d.f}$	784.0a.c,e	795.1$^{a.c.e}$	516.6$^{(*)}$
90 min	1005$^{(*)}$	2085$^{(*)}$	833.2$^{(*)}$	697.1$^{(*)}$	880,6$^{a.b.c,d}$	867.7$^{a.b.c.d}$	527.8$^{(*)}$
120 min	965.1$^{a.c.d.e.f}$	2163$^{(*)}$	914.1$^{a.c.e}$	785.6$^{(*)}$	947.9$^{a.c.e,f}$	891$^{(*)}$	606.4$^{a.b.c.d.f}$
300 min	1362$^{(*)}$	2254.5$^{(*)}$	976.5$^{a.b.c.f}$	994.5$^{a.b.c.f}$	972$^{a.b.c.f}$	993.2$^{a.b.c.f}$	802.5$^{(*)}$
360 min	1381$^{(*)}$	2452.5$^{(*)}$	985.7$^{a.b.f}$	999.9$^{a.b.f}$	979.2$^{a.b.f}$	997.9$^{a.b.f}$	873.0$^{(*)}$

(*) : Significant difference according to all groups; **a**: According to **DR**; **b:** According to **FR; c:** According to **DR+4 ^{0}C; d**:According to **FF; e:** According to **DRIF; f:** According to **FRIF**; **g:** According to **FFDR**. p<0.05 was considered statistically significant.

Table 7. Results of phenolic content analysis of DR+4 ^{0}C and DR extracts by HPLC

Phenolic Compounds	DR+4 ^{0}C EtE	DR-EtE	DR-WE
		(µg/g Ekstract)	
Gallic Acid	19.91	12.19	21.60
H.Benzoic Acid	177.48	112,74	ND
Protocatechuic Acid	53.136	13,22	ND
Catechin	428.53	661.42	125.26
Epicatechin	ND	ND	93.40
Rutin	111.31	121,18	7.62
Quercetin	113.65	132.01	75.33
P Coumaric acid	ND	ND	14.01

ND: Not determined.

Results of Antimicrobial Activity: Antimicrobial activity was detected in all aqueous and ethanolic extracts obtained from FF, DR+4^{0}C, DR and FFDR fruits of rosehip samples. The extracts showed antimicrobial activity against, *Streptococcus pyogenes* ATCC 19615, *Staphylus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 43288 strains, while no activity was detected against *Y. pseudotuberculosis ATCC* 911 and of water extracts against *Proteus vulgaris* ATCC 13315 *bacterial* species (Table 8). No antimicrobial activity was observed in all rosehip infusion products.

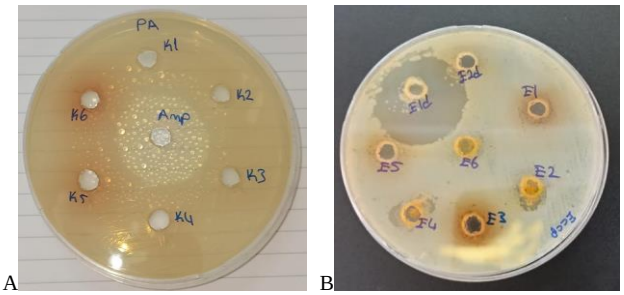

Figure 3. A: Antimicrobial activity in infusion products (*P. aeroginosa*, B: Antimicrobial activity of extracts (*E. coli*).

Table 8. Antimicrobial activity results of different rosehip extracts

Microorganisms	DK Extract		DR +4^{0}C Extract		DR Room Temp.Extract		FFDR Extract	
	EtE	WE	EtE	WE	EtE	WE	EtE	WE
	Zone Diameter (mm)							
Bacillus subtilis	10	-	-	-	12	7	-	-
Staphylus aureus	15	-	6	6	6	10	6	-
Streptococcus pyogenes	6	6	15	-	7	-	11	9
E. coli	30	-	7	7	-	8	17	-
P. aeruginosa	10	-	16	10	6	8	18	12
Proteus vulgaris	-	-	8	-	6	-	17	-
Y. pseudotuberculosis	-	-	-	-	6	-	6	-

DK Extract: Dried rosehip extract frozen and stored at -20 ^{0}C for 3 months
DR Room Temparature Extract: Dried Rosehip storaged at room temparature for 3 monts.
>6 mm zone accepted positive.

DISCUSSION

Herbal teas have an increasing popularity in health-conscious societies due to different properties and factors such as antioxidant, anti-inflammatory, weight loss, sedative effect, sleep problems and cosmetics. (Chandrasekara & Shahidi, 2018; Mieczan & Wójcik, 2024). Tea consumption is widespread throughout the world and is one of the main ways of taking plant bioactive compounds into the body. Traditional infusion methods generally involve the infusion of plant parts (mainly leaves, flowers and fruit parts) at high temperatures. Other studies have shown that the infusion temperature and duration, storage and drying methods of the herbal parts to be infuse significantly affect the antioxidant activity, polyphenolic content and vitamin levels (Akar et al., 2021; Giannakourou & Taoukis, 2021; İlyasoğlu & Arpa, 2017; Saklar et al., 2015).

Rosehip fruits are harvested and used directly from nature by the local people, as well as commercially consumed in the form of whole or fruit pieces or in the form of tea bags. In our study, rosehip fruits were harvested directly from nature and all infusion processes were carried out at a constant temperature of 90 ^{0}C. Antioxidant activity was measured by FRAP, DPPH and ABTS methods which are the most widely used methods. In our study, antioxidant activity, TPC and TFC values increased in all infusion groups from 30th minute to the end of 360th min. There was a marked slowdown of activity in the groups after 300 minutes. The main criteria determining antioxidant activity and phenolic content during infusion are temperature and infusion time (Mieczan & Wójcik, 2024). Akar et al., (2021) examined the antioxidant activities (by FRAP, ABTS and CUPRAC methods) and total phenolic content (TPC) of rosehip tea bags at 7 different temperatures (between 0-90 ^{0}C) and showed that the highest activity (FRAP and ABTS) and TPC values were at 90 ^{0}C. İlyasoğlu & Arpa, (2017) reported that the most suitable infusion temperature in terms of TPC and FRAP values of rosehip tea was around 84-86 ^{0}C with an infusion time of

8-10 minutes. Yildirim et al., (2017) measured TPC, TFC and anthocyanin contents and DPPH antioxidant activity of rosehip tea bags by infusing them at 60, 75 and 90 ^{0}C for 6 time intervals (between 2-12) in their study modelling the effects of infusion temperature and time on bioactive compound content of various tea bags. They found that TPC, TFC, anthocyanin content and DPPH activity of rosehip tea bags increased with increasing temperature and infusion time. These results are consistent with our findings. However,

antioxidant capacity and TPC and TFC may vary depending on the infusion temperature and time according to the plant variety (Banerjee & Chatterjee, 2015; Mieczan & Wójcik, 2024).

Although dried herbal teas are more frequently used, rosehip tea is consumed in a way that includes both dried and fresh fruits (Wenzig et al., 2008). Rosehip fruits are mostly stored dried like other herbal teas and drying processes, storage conditions and durations can affect the phytochemical and antioxidant properties of the fruits (Gruszecki et al., 2024).

In this study, 8 phenolic compounds were detected in phenolic content analyses of ethanolic and aqueous rosehip extracts by HPLC (Table 6). Catechin was found to be a phenolic compound with a very high value in ethanolic extracts compared to other compounds and catechin value in DR EtE was higher than the ethanolic rosehip extract dried and kept at 4^{0}C (DR+ 4^{0}C). These findings are consistent with the view that catechin is one of the dominant phenolic compounds in rosehip fruits and with studies with similar findings (Butkevičiūtė et al., 2022; Turkben et al. 2010). Catechin is a phenolic compound included in the flavonoid group and takes a long time to dissolve in water and its dissolution increases with temperature (Saklar et al., 2015). These properties may explain to a certain extent the higher content of TFC than TPC due to the high catechin values we detected in our study and the increase in antioxidant activity during infusion. It is stated that the number of -OH (hydroxyl) groups and structural configurations of phenolic compounds are positively correlated with their antioxidant activity (Cabrera et al., 2006). Among phenolic compounds, catechins have the ability to scavenge free radicals and form chelates with metal ions, which directly determine their antioxidant activity due to their -OH groups and these -OH groups are capable of capturing free oxygen and nitrogen species in the environment (Bernatoniene & Kopustinskiene, 2018).

In our study, antimicrobial activities of aqueous and ethanolic extracts of rosehip fruits were determined. Gram (+) and Gram (-) strains of microorganisms were used in the study by disc diffusion method which is widely used in other studies. The extracts showed activity against microorganisms other than *Y. pseudotuberculosis* ATCC 911, whereas no activity was observed against *Proteus vulgaris* ATCC13315 in aqueous extracts. The choice of extraction method and solvent, the collection of fruits from different regions and at different times, the fruit content, and the state of the fruit (whole or shredded) can all affect the results. (Hacioğlu et al., 2017; Miljković et al., 2024; Tastekin & Çiftci, 2023). In one study, no antimicrobial activity was observed in methanolic extracts of rosehip fruits collected from Erzurum region against *E. coli*, *B. Substilis* and *S. aureus* strains (Yılmaz & Ercişli, 2011), whereas activity against these strains was detected in most of the ethanolic extracts in our study (Table 7). Taştekin and Çiftci (2023), demonstrated antimicrobial activity against *E coli* and *Staphylococcus aureus* strains in dried rosehip extracts collected from 10 different points in the Samsun Region and homogenised with phosphate buffer. It is stated that polyphenolic compounds may show these effects by forming complexes with water-soluble proteins in the bacterial cell wall or by inactivating some microbial enzymes (Moşanu et al., 2018).

In this study in which disc diffusion method was used, no antimicrobial activity was observed in rosehip infusion products in contrast to the extracts. In a study involving 31 plants, including crushed rosehip fruit tea bags, antimicrobial activity against various microorganisms was detected in the infusion products of rosehip tea bags (Hacioğlu et al., 2017). There are also studies on the antimicrobial effects of brewing products of some other fruit teas. In a study on the antimicrobial activity of infusion products of black and green tea, activities of teas infused at different concentrations for 5 minutes were found on *S.aureus* and *P aeroginosa* strains, while no activity was observed in *E.coli* strain (Kadiroğlu & Dıblan, 2017).

CONCLUSION

In this study, antioxidant and antimicrobial activity and phenolic content analyses were carried out in 7 long-term and gradual infusion samples of fresh, dried, frozen, stored at +4 ^{0}C and -20 ^{0}C rosehip fruits. Conclusions, DR and FR infusions products had higher antioxidant activity, TPC and TFC content than the other infusions products, and these activities and contents continued to increase during 360 minutes of infusion at 90 ^{0}C at a temperature below boiling point. However, increased antioxidant activity TPC and TFC content can be obtained from rosehip fruits both frozen, dried and cured at +4 ^{0}C during long infusion process. If freeze storage is planned, thawing, drying and prolonged infusion (more than 120 minutes) of frozen fruits will provide more effective TPC and TFC content. For the yield of

antioxidant activity, frozen rosehip berries (FF, FFDR) had higher values within 120 min than dried and stored at +4 ^{0}C. The fact that the fruits are not fragmented supports the increase of antioxidant activity, TPC and TFC during the long infusion period. Undivided fruit can be used for long-term infusion and will provide higher antioxidant activity after the 120th minute.

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