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EFFECT OF DRYING TEMPERATURE ON THE BIOACTIVE COMPOUNDS CONTENT OF ROSE HIPS (*Rosa canina* L.)

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ABSTRACT

Dog rose (*Rosa canina* L.) is a beautiful ornamental plant that provides fruit with high biological value. To be available throughout the year, rose hips need to be conserved, such as dried and canned. For this reason, a study was undertaken to investigate the effects of drying as the most common method for preservation. Therefore, the influence of variation drying temperature (20, 35 and 68 °C) on the content of flavonoids, total polyphenols, ascorbic acid, carotenoids and antioxidant activity - determined using the free radical source DPPH (2,2-diphenyl-1-picrylhydrazyl) and a test measuring the measuring of compounds to reduce ferric ions Fe^{3+} (FRAP) - of rose hips was the main topic in this study. Drying decreased total polyphenols (from 38.06 mg g^{-1} by fresh fruits to 9.41 mg g^{-1} drying to 20 °C), ascorbic acid (from 2000.5 mg 100 g^{-1} by fresh fruits to 1308.2 mg 100 g^{-1} drying to 68 °C) and flavonoids, the latter only in those dried at 35 °C (0.54 mg g^{-1}), but did not affect the antioxidant activity of DPPH (84.21–85.73%) and FRAP (2.99–3.41 mgTr g^{-1}). The study also showed that the extraction time influenced the antioxidant activity level of fruit infusions for the first time. The antioxidant potential FRAP value increased systematically with extraction time, but the DPPH values of extracts obtained from dried rose hips were not affected by the extraction time. These results indicate that changes in the antioxidant activity of extracts may not be linear and that the choice of the time of evaluation of this activity may determine the results.

Keywords: antioxidant activity, dog rose, extraction time, fruits, infusions

INTRODUCTION

Dog rose (*Rosa canina* L.) is a shrub whose natural range covers almost all of Europe, except its northern areas, North Africa and West Asia [Jürgens et al. 2017, Selahvarzian et al. 2018]. In addition to the ornamental qualities of the plants, flowers and fruits also have a high utilitarian value. The medicinal properties of rose hips were used relatively early on; Columella [1991] in ancient Rome recommended using grated dry fruits for bee diseases. Saint Hildegard of Bingen [2014] recommended using boiled fruit for a weak stomach, but only for healthy people; weak people should eat crushed raw fruits. There is also great interest in using rose hips due to their high biological value [Jabłońska-Ryś et al. 2009, Fan et al. 2014, Iancu et al. 2020]. Rose hips have a high antioxidant potential compared to many other wild fruits and can be used instead of synthetic antioxidants [Olsson et al. 2004, Jabłońska-Ryś et al. 2009, Egea et al. 2010, Fan et al. 2014, Selahvarzian et al. 2018, Daels-Rakotoarison et al. 2022]. The high antioxidant activity of *R. canina* fruit is due to the content of many compounds with such activity [Olsson et al. 2004, Roman et al. 2013, Daels-Rakotoarison et al. 2022]. These compounds have anticancer, anti-mutagenic, anti-inflammatory, antioxidant, and anti-obesogenic activities and the use of rose hips may contribute to reducing the risk of many diseases [Jabłońska-Ryś et al. 2009, Fan et al. 2014, Fattahi et al. 2017, Selahvarzian et

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al. 2018, Yücel et al. 2019, Iancu et al. 2020, Zahara et al. 2020]. However, the availability of the fruit is seasonal, and extending the period of their use is often associated with drying, which may affect its biological value [Sultana et al. 2012, Vidinamo et al. 2020]. Drying is also a frequently used method for preserving rose hips. Previous results indicate that the content of individual components and the antioxidant activity of the obtained raw materials may depend on the drying temperature [Koca et al. 2009, Moldovan et al. 2021]. However, there are no studies analysing the effect of drying temperature on the antioxidant activity of the obtained infusions, even though these fruits are a component of many herbal mixtures and fruit teas. This study aimed to determine the changes in the antioxidant potential of rose hips dried at different temperatures and the antioxidant potential of rose hip infusions depending on the temperature of fruit drying and extraction time.

MATERIAL AND METHODS

The raw material was rose hips (*Rosa canina* L.), collected from the wild in the third decade of September near Lublin (51°28'42" N, 22°61'80" E, central-eastern part of Poland). The fruits were divided into 200 g samples in triplicate and subjected to drying in a heated room at 20 °C (average temperature approximately 20 ±2 °C, shaded place) and a dryer at 35 °C and 68 °C. The drying time of the samples was 12 days, 2 days and 1 day, respectively. Drying conditions were selected to compare drying in natural conditions (20 °C) and drying at a temperature (35 °C) recommended for raw materials sensitive to higher temperatures and higher drying temperatures (68 °C), as they are often used for rose hips drying. After drying, the raw materials were weighed and stored uncrushed at approximately 20 °C until analyses were performed. Fresh raw materials were analysed immediately after harvest. The rose hips were crushed in ceramic mortars before analyses. All analyses were carried out in triplicate. The dried rose hips were also used to prepare the infusion; 2 g samples were poured into 250 mL of hot water (90 °C) and were taken after 15, 30 and 60 min to evaluate the antioxidant activity of the infusions.

Determination of flavonoid content

A spectrophotometric method determined the flavonoid content in the solution after fresh and dried plant material extraction. Extraction was performed with a mixture of solvents, acetone (Chempur, Piekary Śląskie, Poland), hydrochloric acid (250 g L^{-1}, Chempur, Piekary Śląskie, Poland), and methenamine (5 g L^{-1}, Merck, Poznań, Poland). To determine the flavonoid content, 5 g of crushed fruits were prepared and extracted three times. The analysis was performed according to the methodology given in Polish Pharmacopoeia V [1990], and the absorbance was measured using a UV-Vis spectrophotometer model Hitachi U-2900. The absorbance of the solutions was measured at a wavelength of 425 nm using the reference solution. The total content of flavonoids (mg g^{-1} DM) was expressed in terms of quercetin according to the formula:

$$X = \frac{A \times k}{m}$$

where: A – absorption of the solution of the research; k – convection factor for quercetin; k = 8.75; m – the sample with the raw material in g [Polish Pharmacopoeia V 1990].

Determination of total polyphenols

The Folin-Ciocâlteu method with slight modifications was used to determine the sum of phenol content in the rose hips [Singelton and Rossi 1965, Turkmen et al. 2006]. The 5 g of dried, crushed rose hips were weighed for determination, and 50 mL of methanol (Chempur, Piekary Śląskie, Poland) was added. The research material prepared this way was maintained for 30 min under reflux on a water bath. This mixture was extracted three times with methanol (50 mL). For this purpose, 6 mL of distilled water and 0.5 mL of Folin-Ciocâlteu reagent (Chempur, Piekary Śląskie, Poland) were added to 0.1 mL of the methanolic extracts tested, and the whole mixture was mixed and allowed to stand for 3 min. After this time, 1.5 mL saturated sodium carbonate solution was added, and 1.9 mL distilled water was added and placed in a thermostat (40 °C) for 30 min. Absorbance was then measured at 765 nm using the reagent mixture without the extract as a reference. Measurements were made using a Hitachi U-2900 UV-Vis model spectrophotometer. The results were calculated from the standard curve for gallic acid and expressed as mg of phenolic compounds per gram of dry weight performed per gallic acid.

Determination of carotenoids

Analysis of the carotenoid content was carried out for dry plant material. For this purpose, 0.5 g of the material sample was smashed and crushed in a ceramic mortar with a small amount of 80% acetone (Chempur, Piekary Śląskie, Poland), and then 30 mL of 80% acetone was added and left for 1 h at room temperature. Then, the samples were filtered and made up to 50 cm^3. The determination was carried out according to Lichtenthaler and Wellburn [1983].

Determination of ascorbic acid

The content of ascorbic acid was determined in water extracts. For this purpose, a 2 g sample was triturated with water and filtered into a volumetric flask. Then, the volume was topped up with distilled water to 10 mL. The water extract determined the ascorbic acid at room temperature (approximately 25 °C). The ascorbic acid content was determined by reflectometry using a reflectometer (Reflektometer RQflex 2, Merck) and strip tests (Supelco; Sigma–Aldrich) with a detection range for ascorbic acid levels of 25–450 mg L^{-1}.

Determination of antioxidant activity by the DPPH method

The antioxidant activity by the DPPH method, consisting of the colourimetric measurement of the degree of reduction of DPPH free radicals (2,2-diphenyl-1-picrylhydrazyl, Merck, Poznań, Poland), was carried out for fresh and raw materials dried at different temperatures (20, 35 and 68 °C) and for infusions prepared from dry raw materials. Absorbance was measured using a Hitachi U-2900 UV-Vis model spectrophotometer. The absorbance of the solutions was measured at a wavelength of 517 nm using the reference solution (methanol). To determine DPPH activity, 25 mL of methanol was added to a 2 g sample and left for 24 h, then the solution was filtered and used for testing. The analysis was performed using the method given by Yen and Chen [1995]. The results were expressed as the percentage of DPPH inhibition according to the formula given by Rossi et al. [2003].

$$X\% = 100 - (At / Ar \times 100)$$

where: At – absorbance of the test sample, Ar – absorbance of the blank.

For the prepared infusions, the percentage of free radical inhibition was determined for each dried raw material three times at 15, 30 and 60 min after preparation of the infusions.

Determination of antioxidant activity by FRAP method

FRAP antioxidation activity was determined for prepared infusions of dried plant material according to the method given by Thaipong et al. [2006] and Mulugeta et al. [2022] with modifications. In order to analyse the antioxidant activity of the infusions, 5 g of dried fruit samples were weighed, and 250 mL of hot water was added. After 15, 30 and 60 min, 50 µL of the extract was taken to determine the antioxidant activity by the FRAP method. For the analyses, a FRAP (Merck, Poznań, Poland) reagent was prepared to consist of 0.3 M acetate buffer (pH = 3.6; POL-Aura, Zabrze, Poland), 10 mM TPTZ (2,4,6-Tris(2--pyridyl)-s-triazine; Merck, Poznań, Poland) in hydrochloric acid (Chempur, Piekary Śląskie, Poland),) and 20 mM iron (III) chloride hexahydrate (Chempur, Piekary Śląskie, Poland). The prepared FRAP reagent was added 3 mL to the tubes, followed by adding 50 µL of aqueous extracts, and the whole was maintained at 37 °C for 10 min. After that time, the absorbance was measured at 593 nm using a blank containing distilled water. Absorbance was measured using a Hitachi U-2900 UV-Vis model spectrophotometer. The results were read from the formula curve for the Trolox standard solution. The antioxidant capacity of the samples was expressed as Trolox equivalents (mgTr g^{-1} DM).

Statistical analysis

The obtained results are presented as the means and were statistically analysed by ANOVA, and the averages were compared using Tukey's HSD test at the probability level $\alpha = 0.05$. Statistical analyses were calculated with Statistica 13.3 PL software (StatSof Inc., Tulsa, OK, USA).

RESULTS

The different temperatures for rose fruit drying did not cause statistically significant differences in their dry weight (Table 1). Similar results of dry weight of dried rose hips indicate that all analysed samples were dried to a similar level. Raw material containing between 88.05% (20 °C) and 89.84% (68 °C) dry weight was obtained at all drying temperatures. In the case of fresh fruits, the dry matter was obtained at 44.02%.

Table 1. Contents of analysed components in rose hips expressed as dry weight

Drying	Dry weight (%)	Flavonoids (mg g^{-1})	Total polyphenols (mg g^{-1})	Ascorbic acid (mg 100 g^{-1})	DPPH method (100%)	FRAP method (mgTr g^{-1})
Fresh	44.02 ±1.82 b	0.75 ±0.03 a	38.06 ±3.84 a	2000.45 ±137.9 a	84.21 ±1.26	3.41 ±0.32
20 °C	88.05 ±0.07 a	0.73 ±0.13 ab	9.41 ±1.88 b	1487.53 ±179.0 ab	84.16 ±0.65	3.20 ±0.33
35 °C	88.84 ±0.96 a	0.54 ±0.05 b	11.05 ±2.73 b	1617.66 ±90.4 ab	85.73 ±0.19	2.99 ±0.16
68 °C	89.84 ±0.47 a	0.69 ±0.06 ab	10.68 ±1.03 b	1308.23 ±339.9 b	84.86 ±0.76	3.36 ±0.51

Means followed by the same letter in the columns do not differ significantly by the Tukey test at 5% probability.

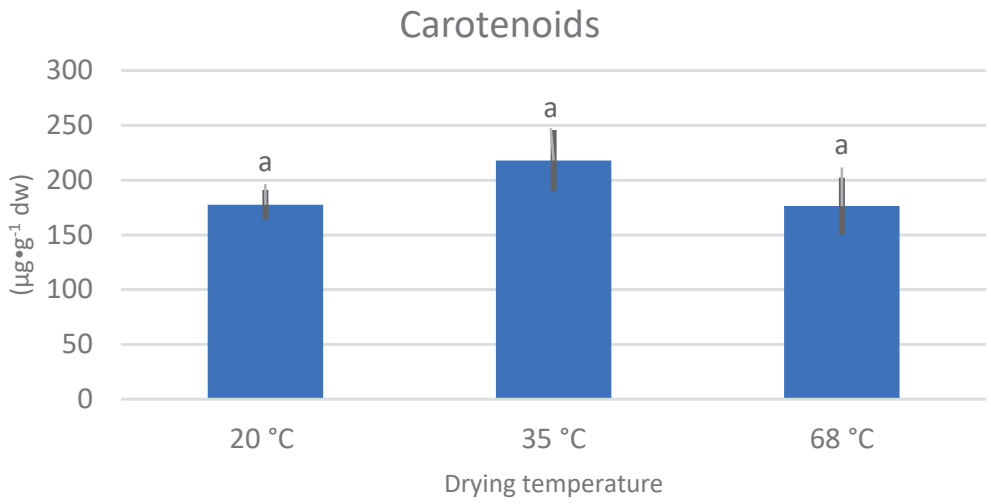

Fig. 1. The carotenoid content in rose hips depending on the drying temperature. The vertical black lines represent the standard deviation

For flavonoid content, fresh and dried fruit at 20 °C showed similar contents of these compounds on a dry weight basis (0.75 and 0.73 mg g^{-1}, respectively). However, the lowest content was in the fruits dried at 35 °C (0.54 mg g^{-1}) (Fig. 1).

The highest total polyphenolic content determined by the Folin-Ciocâlteu method (Table 1) was found in fresh fruit (38.06 mg g^{-1}), while the lowest in fruit dried at 20 °C (9.41 mg g^{-1}). However, different rose hip drying temperatures did not significantly differentiate the total content of polyphenolic compounds (Table 1).

The fresh rose hips contained more ascorbic acid than dried ones (Table 1), but statistically significant differences were found only when comparing fruit dried at 68 °C. Fruit dried at this temperature also had lower ascorbic acid content than fruit dried at other temperatures.

The carotenoid content of the dried raw material did not differ according to the drying temperatures (Fig. 1). The highest content was determined in fruit dried at 35 °C (245.2 µg g^{-1}), while the lowest content was determined at 68 °C (196.3 µg g^{-1}).

Antioxidant activity

Antioxidant activity determined by the DPPH method showed a high antioxidant potential for fresh and dried fruit, ranging from 84.16–85.73%. The drying conditions significantly affected the antioxidant activi-

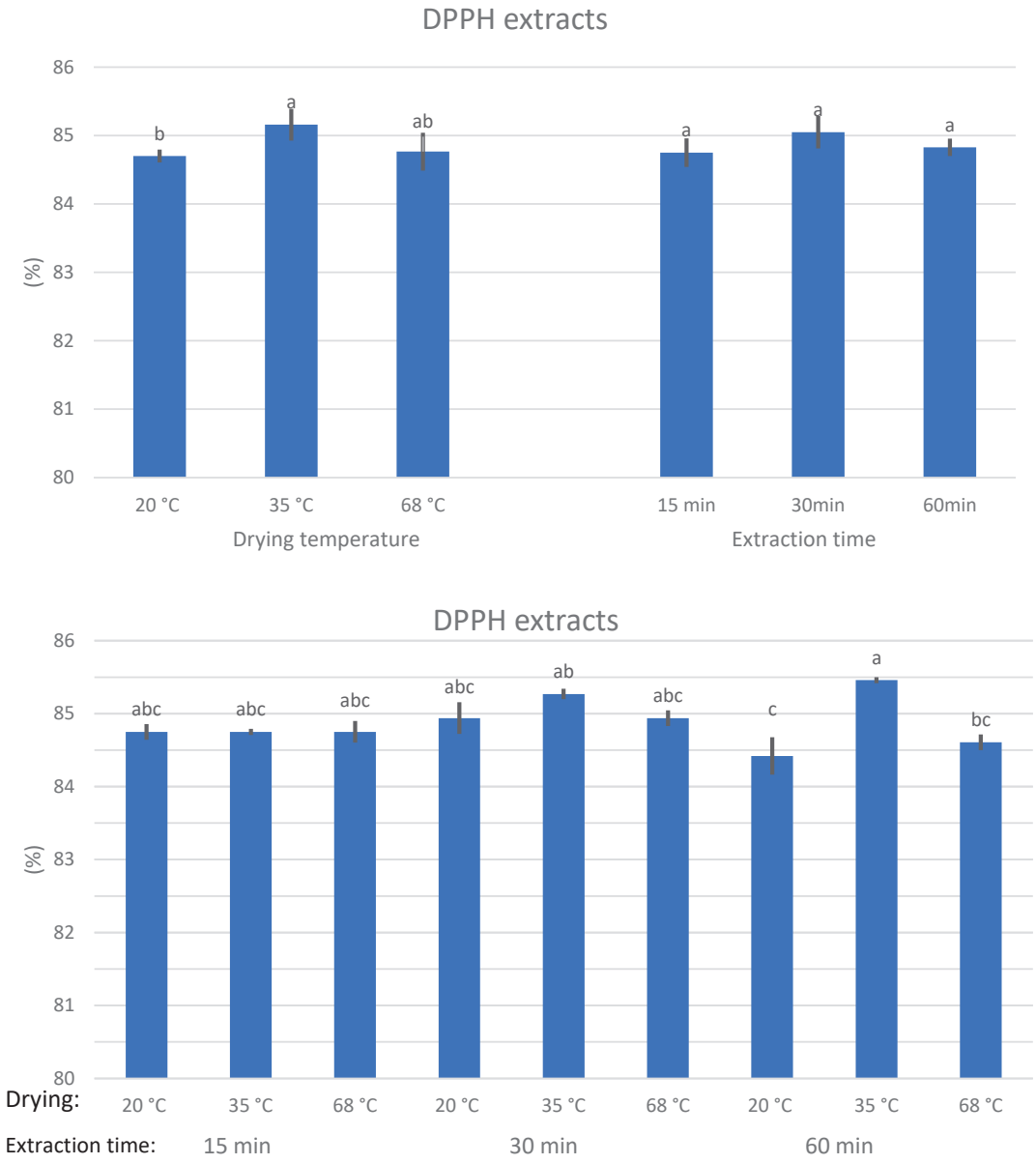

Fig. 2. Antioxidant properties expressed as DPPH (% reduction of DPPH radicals) for extracts prepared from dried fruit measured after 15 min, 30 min and 60 min. The vertical black lines represent the standard deviation

ty, as the lowest was found when the raw material was dried at 20 °C and the highest at 35 °C (Table 1). The antioxidant activity of water rose hip extracts varied depending on the drying temperature. This activity was the highest in the infusions obtained from the fruits dried at 35 °C and the lowest in those dried at 20 °C (Fig. 2). No effect of the extraction time on the antioxidant activity of the infusions determined by the DPPH method was found. In the FRAP reagent method, the lowest antioxidant activity of 2.99 mgTr g^{-1} was found for the fruit dried at 35 °C, while the highest value of 3.41 mgTr g^{-1} was recorded for fresh

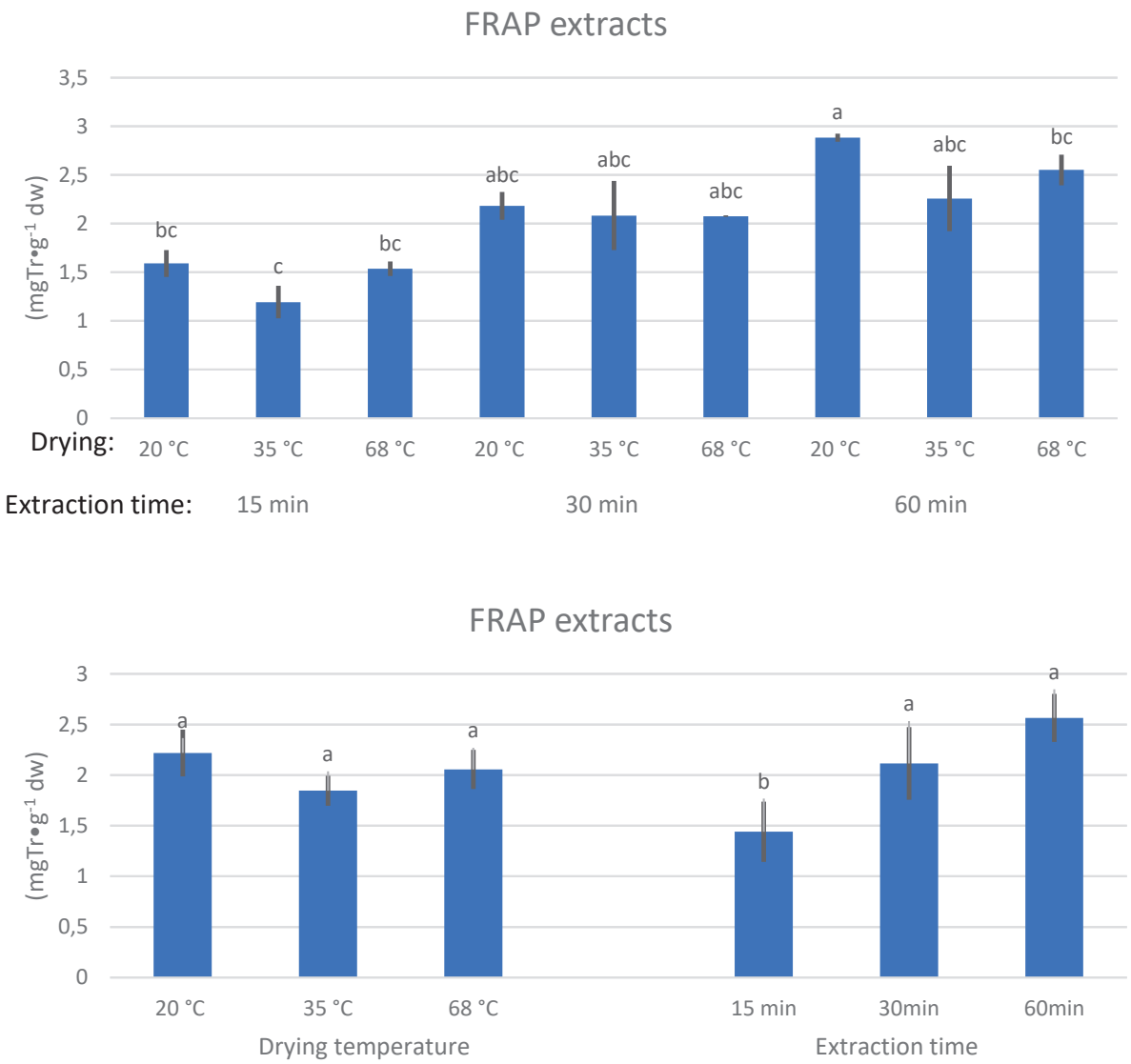

Fig. 3. Antioxidant properties determined by the FRAP method (mgTr g^{-1}) for extracts prepared from dried fruit measured after 15 min, 30 min and 60 min. The vertical black lines represent the standard deviation

raw material, but these differences were not statistically significant (Table 1). In the case of the aqueous extracts obtained from the dried fruit, the extraction time had a statistically significant effect on their antioxidant activity (Fig. 3). The lowest activity, determined by the FRAP method, was obtained for the extracts after 15 min, regardless of the fruit drying temperature. The antioxidant activity determined by the FRAP method increased with the extension of the extract preparation time. The drying temperature of the rose hips used to prepare the extract did not affect their antioxidant activity.

DISCUSSION

Rose hips are characterised by a relatively high dry weight ranging from 38.0% to 68.9% [Márquez et al. 2006, Ercisli 2007, Koca et al. 2009, Türkben et al.

2010, Fan et al. 2014]. Among other reasons, this is due to using fruits with different degrees of maturity for the study [Türkben et al. 2010]. In the experiment, the dry weight of the rose hip was close to the lower of the reported values, at 44.2%, and a similar degree of drying of the raw material was achieved for all drying temperatures.

Drying fruits usually reduces their total phenolic content [Sultana et al. 2012, Nađpal et al. 2016]. It was also confirmed in the present study, while no effect of the drying temperatures on total phenolic content was shown. According to other authors, drying temperature affects the total phenolic content in the rose hip, but the results are inconclusive [Koca et al. 2009, Moldovan et al. 2021]. Koca et al. [2009] showed that with increasing drying temperature, from 50 to 70 °C the total phenolics content decreases; when Moldovan et al. [2021] analysed the same drying temperatures, the highest content of these compounds obtained after drying at 60°C. This may be due to the varying composition of these plant compounds. Nađpal et al. [2016] found, after drying, a decrease in total phenolic content in aqueous extracts of rose hips, while in methanol extract, a decrease in these compounds was not observed. According to Tsai et al. [2002], the drying temperature can influence the composition of phenolic compounds in raw materials. The total phenolic content, in published works, varies widely and ranges from 3.26 mg g^{-1} to 90.6 mg g^{-1} [Olsson et al. 2004, Ercisli et al. 2007, Jabłońska-Ryś et al. 2009, Koca et al. 2009, Roman et al. 2013, Nađpal et al. 2016]. In the work presented here, the total phenolic content ranged from 6.38 to 17.86 mg g^{-1}.

The drying temperature affects the content of flavonoids, and only drying at 35 °C reduced the amount of these compounds in rose hips. The effect of drying temperature on the flavonoid content of rose hip was also noted by Moldovan et al. [2021]. Drying most often decreased the content of flavonoids in the fruit, but the persistence of these compounds' content at the fresh fruit level was also noted [Nađpal et al. 2016, Paunović et al. 2019]. This may be due to the variation in this group of compounds, e.g., the sum of methanol-soluble flavonoids in *R. canina* after drying did not change much when a significant decrease in flavonoid content was recorded in *R. arvensis* [Nađpal et al. 2016]. In the experiment, the flavonoid content ranged from 0.54 to 0.73 mg QE g^{-1} in dried fruit and 0.75 mg QE g^{-1}. The content of flavonoids in *R. canina* fruit most often ranges from 0.2–1.5 mg QE g^{-1} [Adamczak et al. 2012, Roman et al. 2013, Elmastaş et al. 2017]. However, a higher content of these compounds, from 38.52 to 26.47 mg QE g^{-1} DM, was also found [Paunović et al. 2019].

Nađpal et al. [2016] found no differences in ascorbic acid content in aqueous and methanolic extracts obtained from fresh and dried *R. canina* fruit. However, according to other studies, the decrease in ascorbic acid content during the drying process can be as high as 56-65% [Türkben et al. 2010, Paunović et al. 2019]. Koca et al. [2009] obtained lower losses ranging from 20% to 44% of ascorbic acid, which was highest at the lowest drying temperature (50 °C). Similar magnitude losses were obtained in the present study, but these losses were lower when dried at lower (20 °C and 35 °C) than at higher temperatures (68 °C). Dried fruits of *R. canina* are a rich source of ascorbic acid; the amount of this compound ranged from 1308 to 1487 mg 100 g^{-1}, and in the fresh fruit, it was 2000 mg 100 g^{-1} DM. Similar [Jabłońska-Ryś et al. 2009] and even higher results [Olsson et al. 2004, Koca et al. 2009, Taneva et al. 2016] were obtained by other authors. Most commonly, however, the ascorbic acid content of rose hip ranged from 112.20 to 426 mg 100 g^{-1} [Kazaz et al. 2009, Fan et al. 2014, Nađpal et al. 2016], but even from 3.917 to 31.70 mg 100 g^{-1} was found [Türkben et al. 2010, Tumbas et al. 2011]. Drying, especially at the highest temperature (68 °C), caused a decrease in ascorbic acid content in *R. canina* fruits. In their study, Koca et al. [2009] obtained a different relationship, the ascorbic acid content was highest when dried at 60 °C and 70 °C and lowest at 50 °C [Koca et al. 2009]. Koca et al. [2009] showed that drying rose hip at a temperature of 50 °C compared to higher temperatures (60 and 70 °C) decreases the carotenoid content. In the present study, the drying temperature did not affect the content of carotenoids in rose hips. The content of these compounds ranged from 196.3 to 245.2 μg g^{-1} and was lower than the values, 380–731 μg g^{-1}, determined by other authors [Olsson et al. 2004, Koca et al. 2009].

The antioxidant activity (FRAP value) of fresh (3.41 mM Tr g^{-1} DM) and dried (2.99 to 3.36 mM Tr g^{-1} DM) rose fruits did not differ significantly.

Different results were obtained by Koca et al. [2009] in their study: fruit dried at 70 °C had lower FRAP values than those dried at 50 and 60 °C. They also found a significant decrease in FRAP values in the dried compared to the fresh fruits. However, in a study comparing FRAP values in aqueous extracts obtained from fresh and dried *R. canina* fruits, no significant differences were found, in contrast to methanol extracts, where lower antioxidant activity was found in dried fruits [Nađpal et al. 2016].

Radical scavenging activity (DPPH) in fresh and dried fruits was at similar levels regardless of the drying temperatures. Nađpal et al. [2016] found higher radical scavenging activity (DPPH) of dry than fresh fruit in methanolic extracts, but these differences were not statistically significant in aqueous extracts. However, Moldovan et al. [2021] found significantly higher antioxidant activity in rose hips dried at 70 °C than at lower temperatures. Most often, DPPH decreases in fruit after drying, and the magnitude of this decrease depends on the species and drying method, but sometimes, after drying, an increase in DPPH levels in fruit is also recorded [Sultana et al. 2012].

Rose hip infusions showed significant antioxidant activity, which is consistent with the results obtained by Piljac-Žegarac et al. [2010], Tumbas et al. [2011] and İlyasoğlu and Arpa [2017]. When determining antioxidant activity, the extraction time can be of great importance. For the FRAP value, increasing the extraction time increased the amount of compounds determined. However, in the case of DPPH, such a relationship was observed only up to an extraction time of 30 minutes. At the longest analysed reaction time, the effect of drying methods was already more pronounced, with an increase in the DPPH antioxidant activity recorded only in the extracts obtained from fruit dried at 35 °C. In extracts from fruits dried at 20 and 68 °C, the antioxidant activity was already lower concerning drying at 35 °C compared to shorter extraction times of raw materials obtained with the same drying temperatures. İlyasoğlu and Arpa [2017] obtained the highest FRAP value from infusions obtained after adding water at 90 °C, and this water temperature was also used in the presented experiment, compared to 70 and 80 °C. İlyasoğlu and Arpa [2017] obtained different results than those presented in this paper, but their analysis's extraction times were shorter. The highest FRAP value was obtained with the shortest brewing time (2 min), and infusions brewed for 6 and 10 minutes were characterised by lower antioxidant activity.

CONCLUSIONS

Rose hips are a valuable source of antioxidant compounds. The study showed that fresh and dried rose fruits have high antioxidant potential, even though dried *R. canina* fruit showed a lower content of polyphenolic compounds, ascorbic acid and flavonoids depending on the drying temperature. It may indicate the action of other compounds with such an effect or the formation within these groups of compounds with more potent antioxidant activity. Fruits dried at 35 °C showed the highest content of polyphenolic compounds, carotenoids, ascorbic acid and antioxidant activity as determined by the DPPH method, making it possible to conclude that 35 °C is the optimum temperature for drying rose hips with high antioxidant potential.

The extraction time affects the level of antioxidant activity of the tested samples. In the FRAP value case, the antioxidant activity level increases with the extraction time. On the other hand, according to DPPH, the increase in antioxidant activity may have a definite time interval followed by a decrease. However, the results may differ significantly even for the same raw material dried under different temperatures after the same extraction time. These results have a practical aspect; they may suggest the optimal time of ingestion of herbal infusions to be approximately 30 minutes after brewing and later ingestion may be associated with a loss of some of the compounds. However, drawing broader conclusions regarding changes in antioxidant activity depending on the method of fixing the raw materials and the optimal extraction time requires further research.

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