

The effect of air and freeze drying on the content of flavonoids, β -carotene and organic acids in European dog rose hips (*Rosa L. sect. Caninae DC. em. Christ.*)

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S u m m a r y

This paper presents the results of a study on the effect of air drying (at a room temperature) and freeze drying on the content of flavonoids, β -carotene and organic acids in total weight of hips (hypanthia and achenes) of roses from the section of *Caninae DC. em. Christ.* The obtained results show a significant effect of drying conditions on the content of β -carotene and organic acids. In the lyophilized material more organic acids were found, but less β -carotene, than in the rose hips dried at a room temperature. The largest differences were noted in the case of ascorbic acid. Its content in the lyophilizates was on average 5 times higher than in the air-dried hips and it was on average as much as 1.225 g/100 g of dry weight (DW). The amount of citric acid was higher by only 10%, on average. Large differences were also found for β -carotene. Its content in the freeze-dried material, compared to the air-dried rose hips, was on average lower by 74 mg/100 g DW (43%). In the case of flavonoids, the obtained results were ambiguous.

Key words: *Rosa sp., medicinal plants, drying, organic acids, flavonoids, carotenoids*

INTRODUCTION

Rose hips (*Rosae pseudo-fructus*) contain significant amounts of organic acids, flavonoids, carotenoids, tannins, pectins and other active compounds [1-6]. They are considered to be the richest natural source of vitamin C [7-10]. Flavonoids and organic acids found in rose hips inhibit the oxidation of vitamin C which additionally increases its stability and bioavailability in human organism [4, 7, 10, 11-14].

Until now, rose hips were used primarily as vitamin raw material [7, 11, 15]. More recent research indicates their antibacterial, antifungal, antiinflammatory, and even anticancer properties [16-20]. The antioxidative activity of rose hips has also been demonstrated [13, 21-23].

The raw material in question can be obtained from different species [24, 25]. Due to its frequency of occurrence, it is usually dog rose (*Rosa canina* L.), although hips of other taxa of this genus are also considered to be equally valuable [6]. However, some authors stress that there is a great interspecific variation in vitamin C content. According to some older studies, the largest amount of ascorbic acid is found in rose hips of some species from the *Cinnamomeae* section, much less in those within the *Caninae* section [7, 12, 15]. But Olsson and co-authors [3] emphasise that, among the rose species, particularly the taxa from the *Caninae* section contain large amounts of vitamin C, carotenoids and other antioxidants.

In Poland most species of the genus in question belong to the *Caninae* section, including the most common one – dog rose. These are polyploids of hybrid origin, characterised by large variability [26-30]. Due to difficulties with classification and identification of particular taxa, herbal raw material is obtained from various species, which causes it to be non-uniform.

Raw material drying and processing conditions also significantly affect the level of active compounds in rose hips. However, there are few studies in this area and they mainly relate to ascorbic acid [1, 2, 10, 31-34].

The aim of the present study was to determine the effect of the process of lyophilization and air drying (at a room temperature) on the content of flavonoids, β -carotene and organic acids in total weight of hips (hypanthia and achenes) of selected rose species from the *Caninae* section.

MATERIAL AND METHODS

In the study, 37 samples of rose hips were used, representing 8 species from the section of *Caninae* DC. em. Christ. and their 3 hybrids. These were as follows: *Rosa canina* L., *R. dumalis* Bechst., *R. glauca* Pourret, *R. jundzillii* Besser, *R. rubiginosa* L., *R. sherardii* Davies, *R. tomentosa* Sm. and *R. villosa* L. as well as *R. canina* \times *R. dumalis*, *R. canina* \times *R. jundzillii* and *R. sherardii* \times *R. rubiginosa*. The samples were collected in the regions of Greater Poland, Lower Silesia and Lubusz Land (Poland) in 2007. The material was mainly derived from wild specimens, and in several

cases – from the shrubs which are in the collection of the Institute of Dendrology, Polish Academy of Sciences in Kórnik near Poznań.

Each sample was divided into two parts and subjected to lyophilization or room-temperature drying. Air drying was carried out in a closed room, at a temperature of up to 21°C and relative humidity of 50–60%. A total of 258 analyses of active compounds were performed, including 74 analyses for both ascorbic and citric acid, 72 analyses for flavonoids, and 38 analyses for β -carotene.

The content of ascorbic and citric acids was determined using the HPLC-DAD technique after extraction with water with addition of 4% L-cysteine as the stabiliser [35]. The total content of flavonoids (flavonol glycosides) was determined based on the spectrophotometric determination of aglycones using Christ-Müller's method, converted into quercetin after extraction from the material [36]. The β -carotene content was quantified by the external standard method using the HPLC with a diode-array detection at $\lambda=450$ nm [37].

In statistical analysis, t-test for dependent samples was applied, using Statistica 7.1 software [38]. The Shapiro-Wilk test was used to evaluate the normality of distribution of differences between groups, in justified cases excluding outlier observations. The content of flavonoids and β -carotene was given in mg/100 g, whereas the content of ascorbic and citric acids in g/100 g of dry weight of rose hips.

RESULTS

The present study shows that the method of raw material preparation has a significant effect on the content of β -carotene and organic acids in hips of different rose species from the *Caninae* section (fig. 1, 2). In the case of flavonoids, the obtained results are ambiguous (fig. 3-5). In the material subjected to lyophilization, statistically significantly more organic acids were found, but less β -carotene, than in the hips dried at a room temperature. The largest differences were noted in the case of ascorbic acid. Its content in the lyophilizates was on average 5 times higher than in the air-dried rose hips and it was as much as 1.225 g/100 g of dry weight (DW). The amount of citric acid was on average higher by only 10%, respectively. Large differences were also found for β -carotene. Its content in the freeze-dried material, as compared to the air-dried rose hips, was lower by a mean value of 74 mg/100 g DW, by as much as 43%, on average.

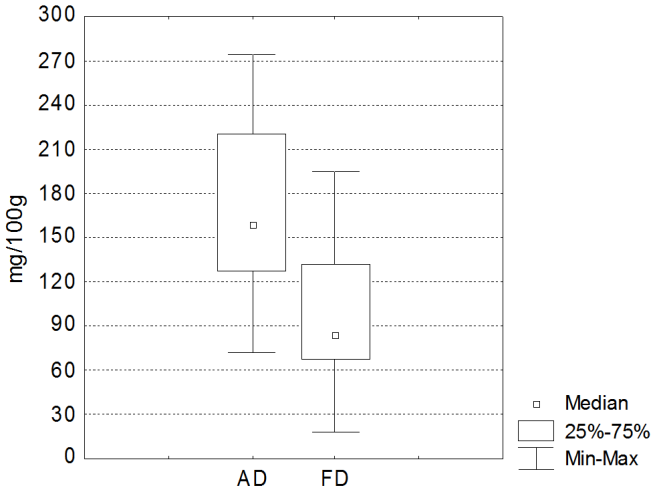


Figure 1. The content of β -carotene in air (AD) and freeze (FD) dried hips of roses from the *Caninae* section. T-test for dependent samples: $t=7.687207$, $p<0.001$, $n=34$

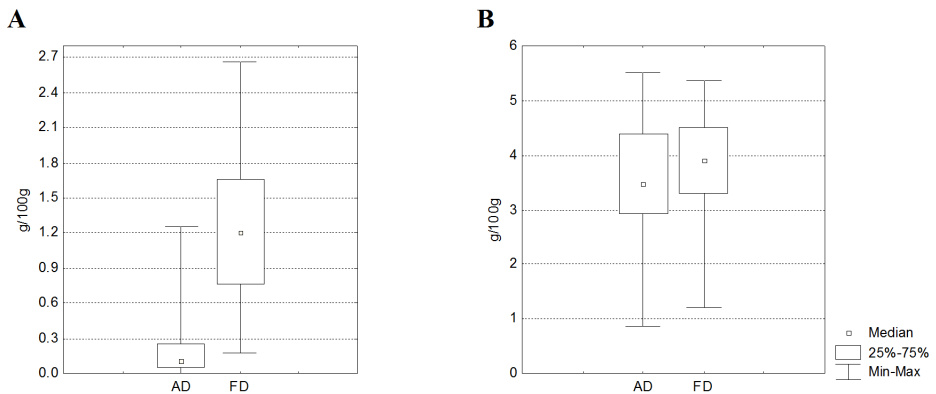


Figure 2. The content of ascorbic (A) and citric (B) acids in air (AD) and freeze (FD) dried hips of roses from the *Caninae* section. T-test for dependent samples: A) $t=-14.0523$, $p<0.001$, $n=74$; B) $t=-3.79082$, $p<0.001$, $n=74$

No statistically significant differences in flavonoids content were found between the samples dried with the use of different methods (fig. 3). Lyophilization (compared to room-temperature air drying) equally frequently resulted both in an increase and decrease in the content of the group of compounds in question (fig. 4). The content of flavonoids in the lyophilized hips accounted, depending on the taxon concerned, for between 67% and 115% of the content of these compounds in the material dried at a room temperature (fig. 5). Such variations in the direction of the effect of the respective drying method were not noted in the case of ascorbic acid (fig. 6) and citric acid (fig. 7), or with regard to β -carotene (fig. 8).

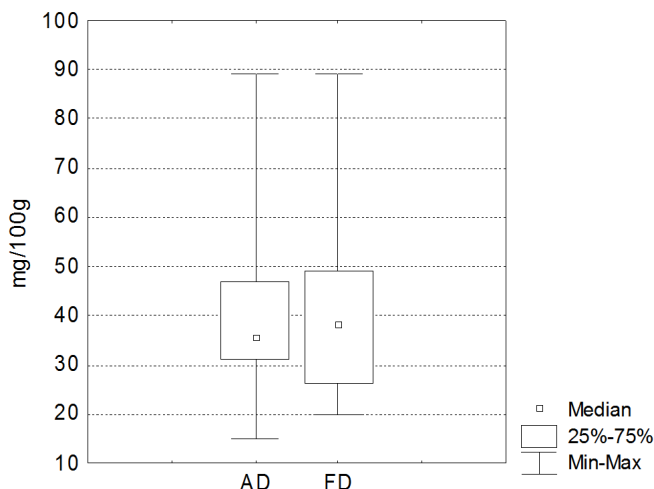


Figure 3. The content of flavonoids converted into quercetin in air (AD) and freeze (FD) dried hips of roses from the *Caninae* section. T-test for dependent samples: N.S., n=68

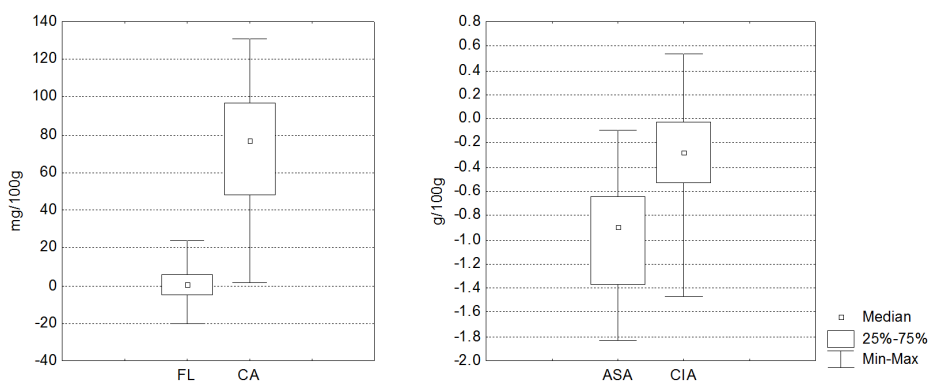


Figure 4. The differences in the content of the investigated compounds in the air and freeze dried hips of roses from the *Caninae* section. (n=250) FL – flavonoids converted into quercetin; CA – β -carotene; ASA – ascorbic acid; CIA – citric acid

In the investigated samples of rose hips, high interspecific variability was found with respect to the content of organic acids (in particular ascorbic acid) and β -carotene, to a lesser degree – with regard to flavonoids (fig. 5-7). For example, for the two most common species: *Rosa canina* and *R. dumalis*, the average content of vitamin C differed in the freeze-dried hips by nearly 2.5 times, and it was 711 and 1729 mg/100 g DW, respectively (fig. 6).

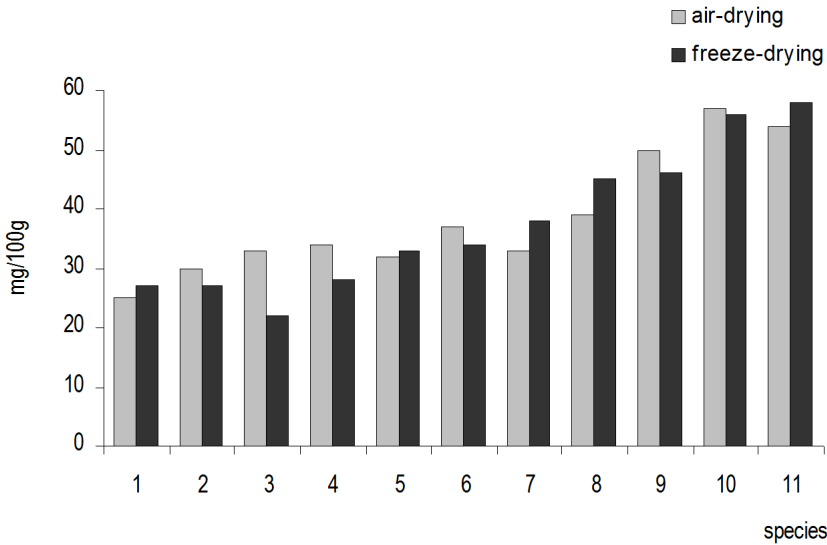


Figure 5. The average content of flavonoids converted into quercetin in air and freeze dried hips of different rose species from the *Caninae* section (n=68). 1 – *Rosa canina* x *R. jundzillii* (n=2); 2 – *Rosa tomentosa* (n=6); 3 – *Rosa canina* x *R. dumalis* (n=2); 4 – *Rosa canina* (n=10); 5 – *Rosa glauca* (n=2); 6 – *Rosa dumalis* (n=12); 7 – *Rosa jundzillii* (n=6); 8 – *Rosa sherardii* (n=10); 9 – *Rosa villosa* (n=4); 10 – *Rosa sherardii* x *R. rubiginosa* (n=2); 11 – *Rosa rubiginosa* (n=12)

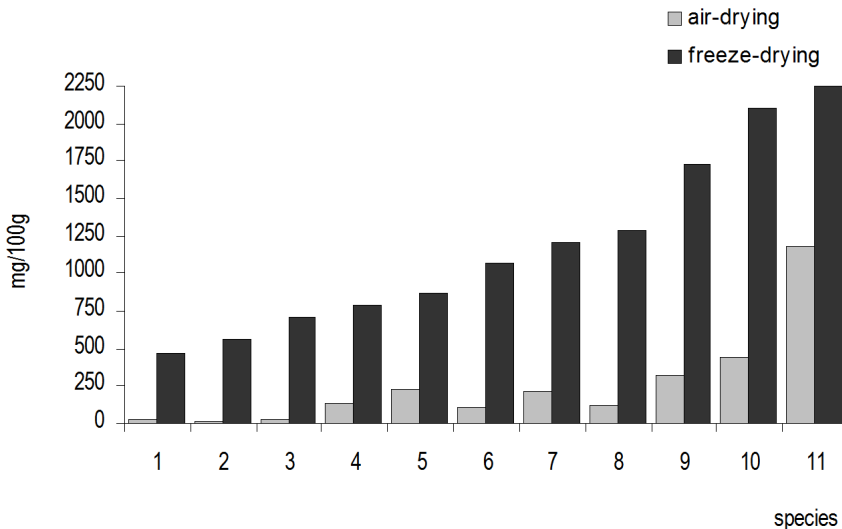


Figure 6. The average content of ascorbic acid in air and freeze dried hips of different rose species from the *Caninae* section (n=74). 1 – *Rosa canina* x *R. dumalis* (n=2); 2 – *Rosa jundzillii* (n=6); 3 – *Rosa canina* (n=10); 4 – *Rosa canina* x *R. jundzillii* (n=2); 5 – *Rosa sherardii* x *R. rubiginosa* (n=2); 6 – *Rosa sherardii* (n=12); 7 – *Rosa rubiginosa* (n=12); 8 – *Rosa tomentosa* (n=6); 9 – *Rosa dumalis* (n=16); 10 – *Rosa glauca* (n=2); 11 – *Rosa villosa* (n=4)

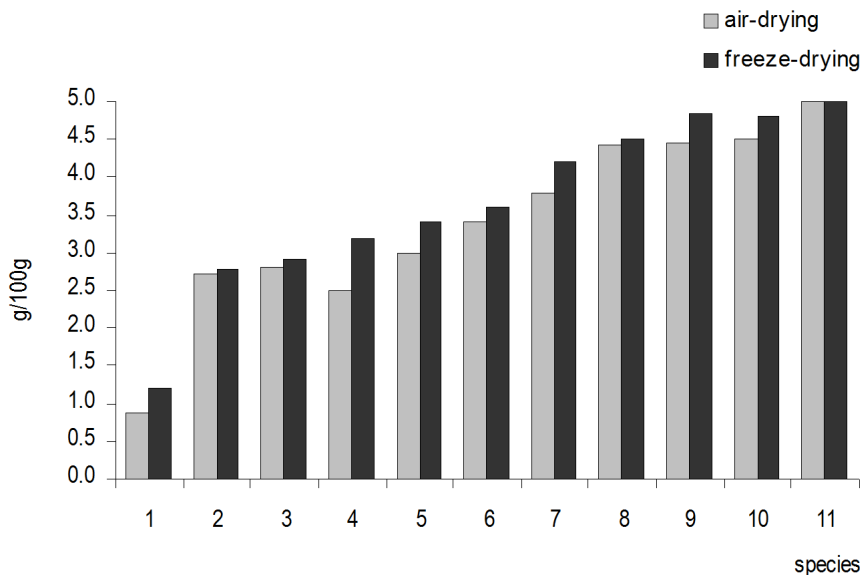


Figure 7. The average content of citric acid in air and freeze dried hips of different rose species from the *Caninae* section (n=74). 1 – *Rosa glauca* (n=2); 2 – *Rosa sherardii* x *R. rubiginosa* (n=2); 3 – *Rosa jundzillii* (n=6); 4 – *Rosa villosa* (n=4); 5 – *Rosa rubiginosa* (n=12); 6 – *Rosa sherardii* (n=12); 7 – *Rosa dumalis* (n=16); 8 – *Rosa canina* x *R. dumalis* (n=2); 9 – *Rosa canina* x *R. jundzillii* (n=2); 10 – *Rosa canina* (n=10); 11 – *Rosa tomentosa* (n=6)

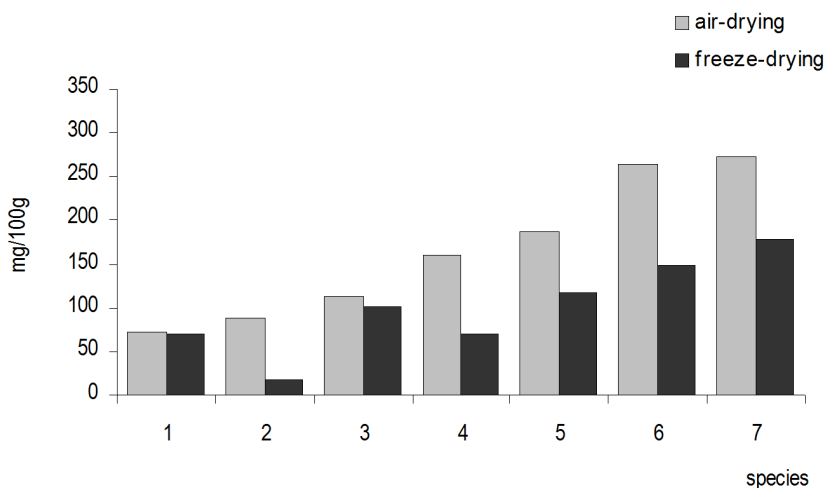


Figure 8. The average content of β -carotene in air and freeze dried hips of different rose species from the section *Caninae* (n=34). 1 – *Rosa villosa* (n=2); 2 – *Rosa jundzillii* (n=2); 3 – *Rosa rubiginosa* (n=2); 4 – *Rosa canina* (n=10); 5 – *Rosa dumalis* (n=14); 6 – *Rosa sherardii* (n=2); 7 – *Rosa glauca* (n=2)

DISCUSSION AND CONCLUSIONS

Rose hips are traditionally dried at a temperature of about 50°C [6, 25, 39] or even 70°C [31]. Some authors recommend two-stage drying, initially at a temperature of 90–100°C, and later on at 50–70°C [12, 40, 41]. These guidelines are formulated primarily having in view the vitamins found in the raw material concerned [12], since air-dried (in natural conditions) rose hips lose up to 90% of vitamin C [41]. At the early stages of drying, when the raw material still contains large amounts of water, many enzymatic reactions occur which result in vitamin C, E, A and β -carotene losses [42]. The antioxidative activity is also reduced [1]. Therefore, it is essential to shorten the process in question to the maximum. However, an increase in drying temperature (from 50 to 60–80°C) results in the acceleration of the vitamin C degradation [33].

In the opinion of Nartowska [43], the process of thermal drying of rose hips should not last longer than 3–4 hours. The study of Strålsjö and co-authors [31] shows that fast drying (for about 2–3 hours) of cut rose hips at a temperature of 70–90°C causes a relatively small (in the order of 3–11%) decrease in the content of ascorbic and dehydroascorbic acids relative to the lyophilization process. The extension of thermal drying (up to 11 hours) reduces the content of the compounds concerned by more than a half. Hence, the long drying time at a room temperature for the raw material in question is the reason for the observed low content of vitamin C. In the present study, it was on average more than 5 times lower than in the lyophilized samples (fig. 2). It is in agreement with the results of Nojavan and co-workers [34]. According to the cited authors, drying of *Rosa canina* hips at a temperature of 15–20°C results, depending on the degree of ripening, in even a 2–6-fold drop in ascorbic acid content compared to samples frozen and stored at –70°C. These adverse changes can be prevented to some extent, since the study of Erentürk and co-authors [32, 33] shows that cutting of rose hips shortens air-drying time, thereby preserving more vitamin C.

The medicinal value of rose hips depends primarily on the content of vitamin C and flavonoids [39]. It is traditionally recommended that flavonoid material should be dried in natural conditions or at a temperature of 120°C [6]. In the opinion of Elbanowska [41], high drying temperature, in the order of 120–150°C, better stabilises flavonoid compounds than 40–60°C. When drying at 37–60°C, the enzymatic hydrolysis of flavonol heterosides occurs. The cited author stresses that, even though the sun's rays cause the decomposition of alkaloids, essential oils and anthocyanins, but they positively affect flavonoid compounds. In this context, it is an interesting fact that in the present study no statistically significant differences were found in the content of flavonoids in the lyophilized rose hips and those dried at a room temperature (fig. 3). The obtained results suggest that drying conditions may affect differently raw material derived from various species (fig. 5). However, the confirmation of this hypothesis requires further research based on larger material. It is possible that the direction and intensity of transforma-

tions of flavonoids (their decomposition or synthesis) in raw material may also be affected by the date of harvesting (the ripening stage) of rose hips. In our climatic conditions, hips of roses from the *Caninae* section ripen from August to October [26], but large intra- and interspecific variations are observed in this respect. Literature data show that during the ripening of rose hips great changes take place in the content of ascorbic acid, polyphenols and carotenoids [2, 34]. The study of Nojavan and co-workers [34] shows that the degree of ripening of *Rosa canina* hips has a significant effect on the differences in vitamin C content between samples dried at a room temperature and samples frozen and stored at -70°C .

Rose hips contain large amounts of carotenoids, mainly lycopene and β -carotene [44, 45]. These compounds have numerous double bonds and that is why they are very susceptible to oxidation, enhanced by light, increased temperature, high pH and the presence of ions of different metals, e.g. Cu^{+2} , Fe^{+2} and Mg^{+2} [46-49]. Preliminary results of the study on *Rosa corymbifera* Borkh. of Novruzov and Shamizade [2] show that at least 50% of carotenoids are decomposed during drying and storage of the rose hips cake. The storage of samples at a room temperature for 30 days results in a loss of up to 25% of carotenoids. The stability of particular compounds from the carotenoid group varies. For example, lycopene can be more susceptible to oxidation than β -carotene [50], although not always [48]. The stability of carotenoids depends to a large extent on the product in which they are [49]. For instance, drying of carrots at 60°C preserves carotenoids (including lycopene and β -carotene) in the same degree as lyophilization [48]. But the obtained results on rose hips drying demonstrate that, in some cases, room-temperature drying can stabilise β -carotene much better than lyophilization (fig. 1).

Natural drying is prolonged and therefore it frequently leads to the decomposition of active compounds [41]. It particularly applies to vitamin C, found in abundance in rose hips. Hence, the shortening of the drying process seems to be an essential requirement in the development of methods for stabilisation of active compounds in the raw material in question. The application of thermal or freeze drying seems to be optimal. According to Gao and co-authors [1], lyophilization and drying at a temperature of $50\text{--}70^{\circ}\text{C}$ stabilise the antioxidative activity in rose hips at a high level. Many authors indicate the advantages of freeze drying, in particular compared to other traditional methods [51]. It is emphasised that lyophilization preserves well the flavour, appearance, colour, texture and biological activity of products [52]. On the other hand, lyophilization is 4–8 times more expensive than other (traditional) drying methods [51, 53, 54]. In this context, it was interesting to conduct this study on air drying (at a room temperature) and freeze drying. The first of these methods may seem to be the least effective, and the other one – the most effective in the stabilisation of active compounds found in herbal raw materials. The obtained results demonstrate that lyophilization is not a universal and optimal method of rose hips drying for all groups of compounds and in some cases it may be distinctly inferior to room-temperature drying.

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WPLYW SUSZENIA POWIETRZNEGO I SUBLIMACYJNEGO NA ZAWARTOŚĆ FLAWONOIDÓW, β -KAROTENU I KWASÓW ORGANICZNYCH W PSEUDOOWOCACH RÓŻ Z SEKCJI *CANINAE* DC. EM. CHRIST.

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Streszczenie

W niniejszym artykule przedstawiono wyniki badań dotyczących wpływu suszenia powietrznego (w temperaturze pokojowej) i sublimacyjnego na zawartość flawonoidów, β -karotenu i kwasów organicznych w całkowitej masie owoców rzekomych (hypancjach i niełupkach) róż z sekcji *Caninae* DC. em. Christ. Uzyskane wyniki wskazują na istotny wpływ warunków suszenia na zawartość β -karotenu i kwasów organicznych. W surowcu poddanemu liofilizacji występowało więcej kwasów organicznych, a mniej β -karotenu, niż w owocach pozornych suszonych w temperaturze pokojowej. Największe różnice odnotowano w przypadku kwasu askorbowego. Jego zawartość w liofilizatach była przeciętnie ponad 5-krotnie większa niż w pseudoowocach róż suszonych powietrznie i wynosiła średnio aż 1.225 g/100 g suchej masy (s.m.). Odpowiednio ilość kwasu cytrynowego była przeciętnie większa jedynie o 10%. Duże różnice stwierdzono także dla β -karotenu. Jego zawartość w surowcu liofilizowanym, w stosunku do owoców rzekomych suszonych powietrznie, była niższa średnio o 74 mg/100 g s.m. (43%). W przypadku flawonoidów uzyskane wyniki były niejednoznaczne.

Słowa kluczowe: *Rosa* sp., rośliny lecznicze, suszenie, kwasy organiczne, flawonoidy, karotenoidy