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Rosehip extraction: Process optimization and antioxidant capacity of extracts

Research Article

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Abstract: This article examines the extraction of rosehip to study the recovery of a number of compounds with antioxidant properties (polyphenols, flavonoids, and β-carotene). Two varieties of rosehip, cultivated and wild are used as raw material. A detailed study of the process kinetics at different operating conditions is carried out in order to determine appropriate processing parameters, which results in extracts with higher content of target compounds and higher antioxidant capacity. Data on the concentration of active components in the different parts of the fruit (skin, seeds, and pappi) are also obtained, which gives information about their distribution within the fruit. The comparison of wild and cultivated varieties demonstrates the better quality of the cultivated one. The results are useful for production of improved and enriched rosehip extracts with higher content of antioxidant substances that have proven beneficial effects on the human health.

Keywords: *Rosa canina • Process optimisation • Extraction kinetics • Polyphenols • Flavonoids*

1. Introduction

The subject of this study is *Rosa canina L.,* a member of the Rosaceae family. This plant is an important food containing low levels of fat and high levels of vitamins, minerals and fibers [1]. It is generally widespread in nature as a wild shrub and the rosehips are its fruit. The use of Rosa canina as a remedy dates back to the time of Hippocrates. Rosehips have traditionally been used as a vitamin supplement or for health food products in many European countries, because they are a richer source of vitamin C than other commonly available fruits or vegetables [2-4]. They also contain other vitamins (K, P) and minerals (Mg, Fe, Mn, Al) [5,6].

The content of organic compounds in rosehips has also been studied. Many authors have reported the presence of ascorbic acid, phenolic acids, hydrolysable tannins, proanthocyanidins, anthocyanins [7], flavonols (kaempferol and quercetin glycosides) [8], as well as carotenoids, which consist mostly of β-carotene, rubixanthins and lycopene [9].

According to Chrubasik *et al*. [6], various preparations and isolated constituents from rosehip have been studied in a variety of *in vitro* and *in vivo* tests. They have demonstrated anti-oxidant and anti-inflammatory activity, impact on body fat, blood glucose, plasma and biliary lipids, antiulcerogenic and probiotic effects, effects on urine excretion and composition, effects on muscle tone and nerve conduction, and antimicrobial effects. Rosehip seed oil is used to treat eczema, trophic ulcers of the skin, neurodermitis, cheilitis and others.

The primary positive effects of rosehip extracts are due to their antioxidant activity. A number of studies have identified substances with antioxidant activity, primarily polyphenols, flavonoids and β-carotene [10]. However, there are no systematic studies on the selection of appropriate conditions for better extraction of these compounds. The aim of this study is to investigate the extraction kinetics of rosehip fruits at varying conditions in order to select those that maximize the antioxidant content of the extracts.

The majority of existing studies deal with wild rosehip fruits. However, our study is focused on a patented cultivar Plovdiv-1 (Pd-1) from the Higher Institute of Agriculture, Plovdiv, Bulgaria [11]. This variety is grown from seeds,

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has no thorns and bears more hips (about 20) per sprig. The fruits are larger, glossy, and elongated. This cultivar is appropriate for production of flakes from the skins, which are rich in vitamin C. Seeds are used to produce oil. This variety is distinguished for its good fertility and drought-resistance.

This paper deals with extraction from ground whole rosehips, as well as from separated parts of the fruit – skin, seeds and pappi. Another objective is to compare extracts from wild and cultivated rosehips, so as to establish whether the cultivated variety has better properties.

2. Experimental procedure

2.1. Plant material

Rosehips (Pd-1) used in extraction experiments were dry fruits from the Plovdiv region, Bulgaria, harvested in the winter of 2010. The weight of a single dry fruit ranged from 1.7 to 2.4 g (mean about 2 g). The fruits were disintegrated and classified in order to ascertain their relative composition (by weight): skin 36.9%, pappi 3.7%, and seeds 59.4%.

Wild rosehip dry fruits were harvested during the winter of 2011 in the Sofia region, Bulgaria. Their mean weight was 0.75 g. The weight distribution of different parts was: skin 51.4%, pappi 7.6%, and seeds 41%.

Fruits of Pd-1 were more than 2.5 times heavier than the wild fruits. The two varieties differ in the proportion of their parts. Pd-1 contains more seeds, about 37% skin and a small amount of pappi. The main part of wild rosehip was skin, followed by seeds, with the percentage of pappi being twice that of Pd-1.

In some experiments, whole fruits were ground to particles that were smaller than 2 mm [12] and were extracted together. In other experiments, the fruit constituents were separated and each of them was individually extracted. In the latter, the mean dimensions of skin particles was 1.4 mm long and 0.55 mm thick, while the seeds and pappi were powdered to mean size 0.2 mm.

2.2. Chemicals used

Folin-Ciocalteu reagent (2N solution, Sigma), gallic acid (Sigma), dehydrated Na_2CO_3 (Valerus), ethanol (96%, Valerus), DPPH (2,2-Diphenyl-1-picrylhydrazyl, Sigma), methanol (99.9% Lab Scan) were used to analyse the polyphenols and determine the antioxidant capacity of the extracts. Quercetin, β-carotene and all other necessary chemicals were manufactured by Sigma–Aldrich.

2.3. Extract preparation

All extracts except those used to find the appropriate solid/liquid ratio were prepared in an identical manner: 5 g of dry and ground plant material were mixed with 50 mL of solvent (water, 96% ethanol or their mixtures) in a thermostatic shaking device. Runs at different temperatures were carried out.

2.4. Analytical methods

The samples of extracts were analyzed for total polyphenolic content (TPC), concentration of flavonoids, β-carotene and antioxidant capacity (AOC). Other samples were evaporated at mild temperature (105°C) until they reached a steady weight, so as to determine the quantity of dry solid matter extracted from the plant at particular conditions.

2.5. Determination of total polyphenolic content

The TPC was determined using Folin-Ciocalteu reagent [10]. The treated samples of the extracts acquired coloring proportional to their TPC. They were analyzed with a double-beam UV-VIS-spectrophotometer (UNICAM®-Helios β) at 765 nm and were compared to a calibration line obtained with Gallic Acid (GA) as a standard reference.

2.6. Antioxidant capacity (AOC_{AA})

The AOC was determined using the DPPH method, which has a simple preparation procedure, and gives stable and reproducible results. The method is based on neutralization of free radicals emitted by the DPPH solution, resulting in a colored solution [13]. The latter is analyzed spectrophotometrically at 517 nm [14].

An important application aspect of the DPPH method is the difference in antioxidant reaction rates, which vary from very fast to very slow. Thus the overall rate of sample discoloration is a sum or combination of individual antioxidant reaction rates. Consequently, the photometric measurement of discoloration has to be performed after some period of sample retention. Different retention times are recommended in the literature (from 30 to 60 min) [15-17]. A longer retention time (60 min) was applied in this study in order to ensure long enough incubation period.

The capacity of the extract to inhibit free radicals (IC [%]) is calculated by the expression

$$IC\ [\%] = [(A_o - A_s)/A_o] * 100 \qquad (1)$$

where A_o is the absorption of a control sample (methanol), and A_s is the absorption of an extract sample.

Table 1. Yield at different liquid/solid ratios (hydromodules).

Hydromodule	Yield (mg g^{-1})		
	Polyphenols	Flavonoids	β-carotene
5	25.6	0.9	0.2
6.7	28.4	1.0	0.2
10	35.1	1.5	0.2
15	35.0	1.5	-
20	34.9	1.5	0.2

The antioxidant capacity is often presented as the IC50% value, which represents the concentration of a sample that inhibits 50% of a standard initial DPPH amount [13]. This value can be determined from the relationship that expresses IC as a function of extract concentration C.

The graph of IC(%) as a function of C was also constructed based on photometric measurements on a series of samples that contained varying amounts of extracts (expressed as ml of extract in one liter of solvent). Each extract was diluted to obtain a linear graph in the IC interval from 0 to more than 50%. Then, the sample concentration C that reduced 50% of free radicals was calculated from the resulting linear equation, or was simply determined from the graph.

In order to obtain more reliable values for the antioxidant capacity of extracts, the same procedure was applied to a popular antioxidant – ascorbic acid (AA) - vitamin C, and its IC50% value. The antioxidant capacity (AOC_{AA}) was obtained by division of IC50% for the AA solution by that of the extract. The result was expressed as grams of AA equivalent to one milliliter of extract or, after recalculation in mass units, as milligrams AA equivalent to one gram Rosa canina fruit. Ascorbic acid was used as a control antioxidant not only because of its popularity, but also because of its very fast reaction with DPPH (compared, for example, to the slow rate of the DPPH - Gallic acid (GA) reaction, particularly at low concentrations). In addition, AA shows similar antioxidant capacity with different methods (TEAC, ORAC, DPPH) [13,18] while GA shows 3 times greater AOC with TEAC than with ORAC and DPPH [13,19].

2.7. Content of flavonoids

The total concentration of flavonoids was determined using the method of Ordonez and Gomez [20]. Equal volumes of the sample and 2% $AlCl_3$ were mixed and kept at room temperature for 1 hour. The absorbance of the resulting color solution was measured at 420 nm. The total flavonoid content was calculated using a calibration curve for quercetin.

2.8. Determination of β-carotene

β-carotene was analyzed according to the European Pharmacopoeia 6.0 [21] method as follows: the absorbance of a test solution was measured at the maximum absorption wave length of 455 nm, using cyclohexane as a compensation liquid.

3. Results and discussion

The main groups of bioactive compounds with antioxidant properties in rosehip fruits are polyphenols, flavonoids and β-carotene. Our task is to study the extraction kinetics of each one of these substances in order to select optimal conditions for their extraction. At the same time, the antioxidant capacity of the extracts is determined. Therefore, the total concentration of these substances can be correlated with the AOC of the extract.

The extraction efficiency strongly depends on the solvent used. Substances with antioxidant activity are generally of a polar type. That is why polar solvents are commonly used for their extraction. Ethanol and water are among the most widely used solvents due to their non-toxicity. Our study is carried out with ethanol, water as well as with their mixtures.

Most of the results below are obtained with ground fruits of cultivar Pd-1. The data for wild rosehip and for different parts of the fruit of both varieties (skin, seeds, pappi) are marked specifically. The points on the graphs are mean values of 3-4 parallel runs. The maximum deviation of results was 4.43% for polyphenols, 8.06% for flavonoids and 10% for β-carotene. The smaller the measured quantity, the higher the deviation.

As a first step, we have examined the yield of target compounds (mg g^{-1} raw material) at different hydromodules (liquid-to-solid ratios) in order to ensure operation with a sufficient quantity of solvent, thus eliminating potential solubility limitations. Table 1 illustrates these results. The experiments are carried out at the solvent (water) boiling point with Pd-1 ground rosehip fruits for 60 min. The results with other solvents are similar. The contact time is chosen from kinetic experiments (see Fig. 1). According to Table 1, for hydromodules up to 10, more solvent extracts more substance, *i.e.*, the solvent quantity is not yet enough to complete the extraction. At hydromodules more than 10, the extracted quantity does not change, *i.e.*, more solvent does not extract additional quantity. Consequently, a solvent-to-solute ratio of 10:1 was chosen as an appropriate value for the hydromodule.

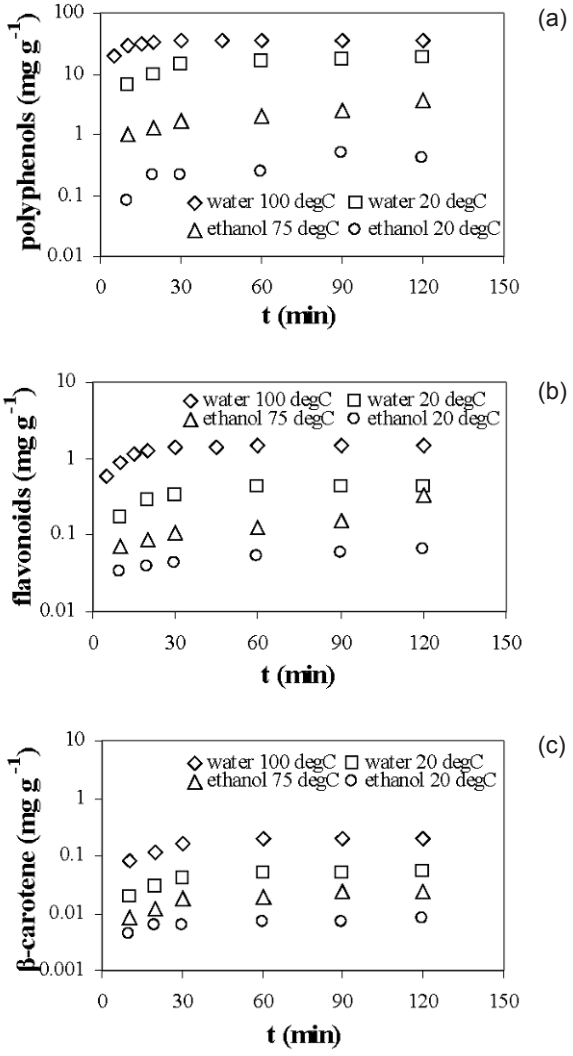

Figure 1. Kinetics of polyphenols (a), flavonoids (b) and β-carotene (c) extraction using water and ethanol as solvents.

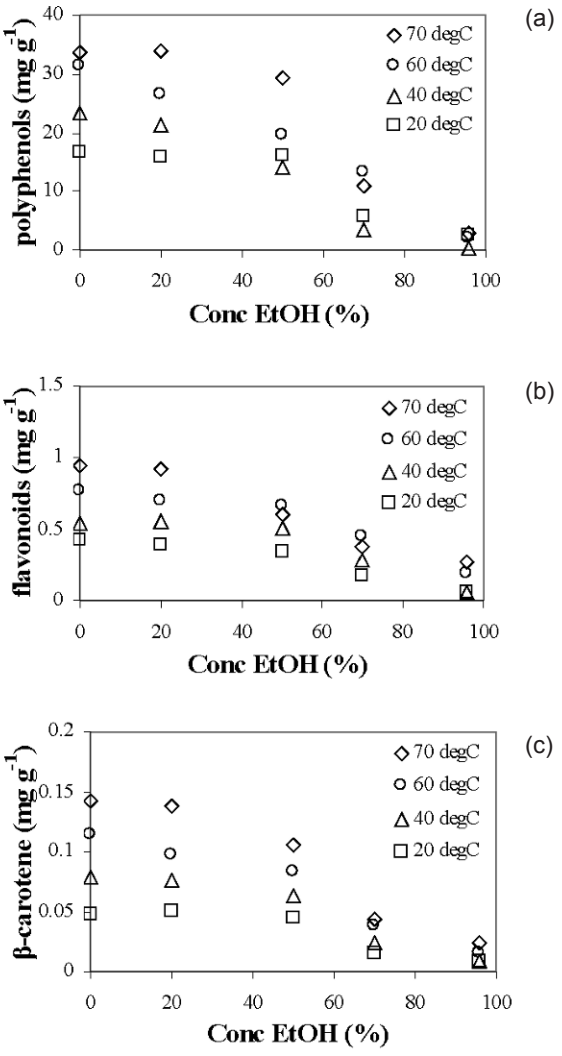

Figure 2. Influence of temperature on the total yield of polyphenols (a), flavonoids (b) and β-carotene (c) extracted with different water-ethanol mixtures.

Figs. 1a-1c present extraction curves as a cumulative yield (mg substance per g raw material) over time for two solvents (water and 96% ethanol) at their boiling points and at ambient temperature.

Generally, the curves have three zones of different shapes. The initial steep section corresponds to the dissolution of easily available substances located along the surface of particles. The rounded part of the curve reflects the simultaneous dissolution of residual substance from the surface and the interior of the particle (zone of mixed control). The slowly increasing asymptotic part corresponds to the dissolution of the substance from internal pores, controlled by the internal diffusion inside the particle.

From Fig. 1 it is obvious that the target substances are much more soluble in water than in pure ethanol (by a factor of approximately 10). In addition, with both solvents the plateau is achieved after about 60 minutes, *i.e.*, the extraction is completed in about an hour.

Figs. 2a-2c illustrate the total yield of polyphenols, flavonoids and β-carotene after 1 hour extraction with different ethanol-water mixtures.

The results show that a higher proportion of ethanol in the solvent reduces the extraction efficiency, *i.e.*, the quantity of target components decreases. The extraction with 96% ethanol is almost independent of the temperature (for polyphenols and β-carotene). The effect of temperature is more pronounced at lower content of ethanol in the solvent.

Because of the lower solubility of target compounds in ethanol or ethanol-water mixtures, only water was used as a solvent in subsequent experiments.

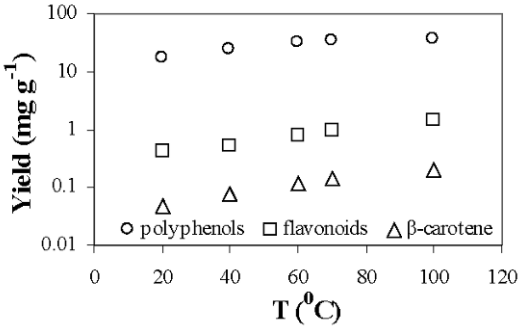

Figure 3. Yield at different temperatures, 1 hour water extraction.

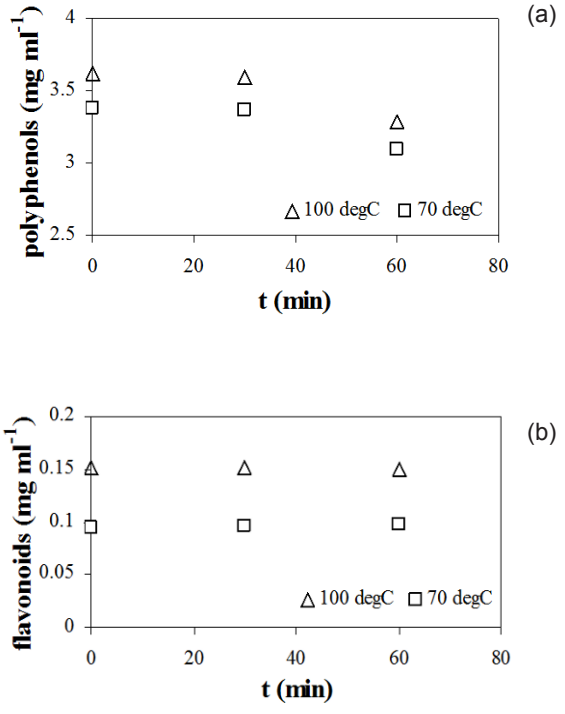

Figure 4. Concentration changes in extracts obtained at 70°C and 100°C in the course of one hour of additional boiling.

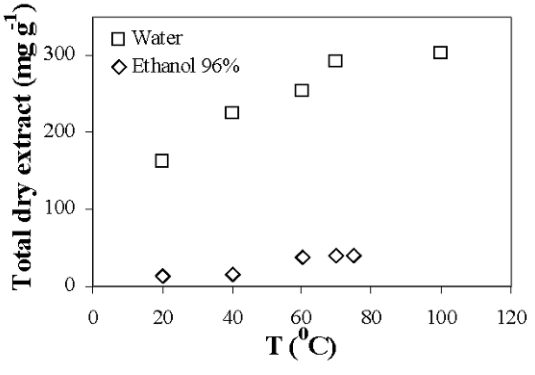

Figure 5. Amount of total dry extract at different temperatures.

Table 2. Content of target compounds in the dried extract (water extraction).

Extraction temperature (°C)	Polyphenols (%)	Flavonoids (%)	β-carotene (%)
20	10.2	0.3	0.03
40	10.3	0.2	0.04
60	12.4	0.3	0.04
70	11.5	0.3	0.05
100	11.6	0.5	0.06

The effect of temperature on the yield after one hour of water extraction is presented in more details in Fig. 3.

Not surprisingly, the solubility of target components improves at a higher temperature. The quantity of polyphenols increases by a factor of 2.3, the quantity of flavonoids by more than a factor of 3, and the quantity of β-carotene by a factor of 5. An interesting behavior is observed with polyphenols, wherein the yield at 70°C and 100°C is almost the same. Consequently, in the case of polyphenols as a sole target component, it is not useful to carry out the extraction at a temperature higher than 70°C. If the objective is the production of a total extract containing more antioxidant substances, 100°C is recommended as the operational temperature (boiling water extraction). Although at this temperature the quantity of polyphenols is not significantly higher than at 70°C, more flavonoids and β-carotene are extracted. So, the total concentration of the studied bioactive substances is higher in the extracts obtained at 100°C.

The comparable yield of polyphenols at 70°C and 100°C might be an indication for eventual thermal destruction at a higher temperature. For this reason, a study on thermal stability of the components present in larger quantities (polyphenols and flavonoids) was carried out. Samples of the liquid extracts obtained at both temperatures after 1 hour extraction were boiled further for one hour.

Fig. 4a gives evidence for thermal degradation of polyphenols, which reduces their quantity by about 10%, while the amount of flavonoids remains at a constant value (Fig. 4b). Thus, prolonged boiling is not favorable to the quality of the extract.

To determine the total amount of extracted matter, samples of liquid extracts were dried until they reached a constant weight. As expected (see Fig. 5), the quantity of total extract increased with temperature and became higher at the boiling point of the respective solvent.

Table 2 summarizes results for the amount of target components in the dried extract.

Table 3. Content of target compounds in cultivated and wild rosehip, water extraction at 100°C.

Compound	Whole fruit (mg g^{-1})		Skin (mg g^{-1})		Seeds (mg g^{-1})		Pappi* (mg g^{-1})	
	Pd-1	Wild	Pd-1	Wild	Pd-1	Wild	Pd-1	Wild
polyphenols	35.1	24.2	78.2	42.9	5.8	5.0	18.8	13.5
flavonoids	1.5	2.2	2.5	3.6	0.6	0.1	1.0	1.0
β-carotene	0.2	0.2	0.3	0.3	0.03	0.03	0.1	0.1

**The amount of various compounds in pappi might be slightly lower than reported, because pappi contain some quantity of finely ground skin.*

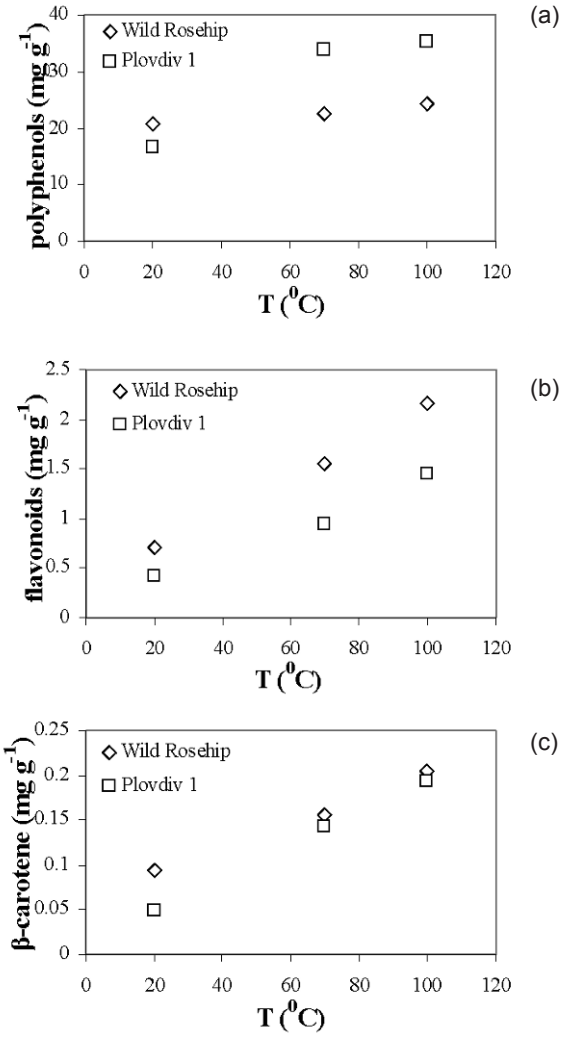

Figure 6. Yield of target compounds at different temperature, 1 hour water extraction.

Although the extracted quantity of polyphenols rises with the temperature (see Fig. 3), its percentage in the dried extract does not significantly change because of the proportional rise of the total extracted mass (see Fig. 5). However, the content of the other substances in the dried extract is almost double when processed at high temperature. Generally, the concentration of target compounds in the dried extract is 3 times higher than that in the fruit itself.

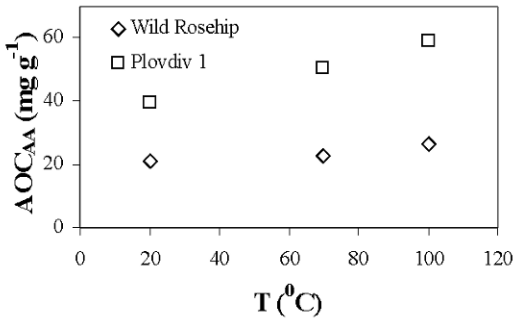

Figure 7. Antioxidant capacity of extracts obtained at different temperatures, 1 hour water extraction.

Figs. 6 and 7 compare extracts from ground fruits of Plovdiv-1 and wild rosehips.

As seen from Figs. 6, water extracts of Plovdiv-1 contain more polyphenols, wild rosehip contains more flavonoids, and both varieties contain equal amounts of β-carotene. The antioxidant capacity of all extracts from Plovdiv-1 is approximately two times higher than that of the wild variety (see Fig. 7).

The overall tendency is that a higher content of target components corresponds to a higher antioxidant capacity. According to Fig. 6a, the polyphenolic content of the extract at 70°C and 100°C is about the same. However, Fig. 7 indicates a higher antioxidant capacity (AOC) of the extract at 100°C. A possible reason might be that flavonoids and β-carotene are better extracted at 100°C, and other compounds with antioxidant activity (like vitamins, minerals *etc.*) are also better dissolved [22], which increases the AOC of the extract.

Table 3 compares both varieties by the amount of target compounds in the whole fruit and in its different parts - skin, seeds and pappi.

According to Table 3, the highest amount of target antioxidant components is found in the skin. The fruits of Plovdiv-1, as well as each of its parts, contain more

polyphenols than the wild rosehip. However, as a whole, wild rosehip contains more flavonoids, although the flavonoid levels in its seeds are about 4.5 times lower than those in Plovdiv-1 seeds. β-carotene is found in equal concentrations in both the fruits and their parts.

When comparing the proportion of target compounds extracted from Plovdiv-1 whole fruits, the main substances are polyphenols, which are found in concentrations 24 times higher than flavonoids; flavonoid levels are almost 8 times higher than β-carotene, and β-carotene levels are over 180 times lower than polyphenol levels.

4. Conclusion

This study on the extraction of a number of substances with antioxidant activity (polyphenols, flavonoids, β-carotene) from rosehip shows that polyphenols are the most abundant compounds in these fruits. Rosehip skin is the richest part of the fruit and the bulk of polyphenols is located in it.

By studying the process kinetics at varying operational conditions, optimized process parameters are determined experimentally, at which the target bioactive substances are more completely extracted, and at which the extracts show the highest antioxidant capacity. Water extraction at the boiling point for one hour produces the extract with the highest antioxidant capacity.

The comparison of cultivated and wild rosehip reveals better properties of the cultivated variety Plovdiv-1.

This paper supplies useful information for improving the extraction process to produce enriched extracts from rosehips with a higher content of antioxidant substances beneficial to human health.

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