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INFLUENCE OF ENVIRONMENTAL FACTORS ON ANTIOXIDANTS CONTENT IN ROSE HIPS

L. POGAČNIK[1] N. POKLAR ULRIH[1]

Abstract: *Total phenolics (TP), antioxidant capacity (AOC) and vitamin C content of rose hips, harvested at ten different locations in Slovenia, were determined in the course of this study. The hips were harvested twice, in September and in late October (with 42 days difference). The determined values vary regarding the location and harvesting season. It was found out that during the ripening in late autumn the concentration of vitamin C decreases, while the TP and AOC increase. During this period, the level of oxidized form of vitamin C (dehydroascorbic acid) also increases, which coincides with the decreased level of ascorbic acid.*

Key words: *rose hips, antioxidants, vitamin C, ripening, environmental factors.*

1. Introduction

The rose hip is the fruit of the rose plant that typically is red-to-orange, but ranges from dark purple to black in some species. Rose hips begin to form in spring, and ripen in late summer through autumn. The botanic name is derived from the common names 'dog rose'. It is known that the plant is high in antioxidants and particularly the hips are noted for their high vitamin C level. They are considered one of the richest plant sources available. However, HPLC analyses of fresh rose hips and several commercially available products revealed a wide range of L-ascorbic acid content, ranging from 0.03 to 1.3% [8].

The fruit is used to make syrup, tea and jam. In the early 1950's a refreshing drink Cockta made of natural ingredients: rose hips, vitamin C, various herbs, spring water, and caramelized sugar was created in Slovenia and it remained one of the most popular soft drinks until now. Nowadays, rose hips can be found on the market also as food supplements in a form of capsules, cream, extracts, syrup, tablets, teas, and tincture in combination with vitamin preparations. A natural source of vitamin C rose hips has been claimed to be useful as a laxative, capillary strengtheners, and boost to the immune system to prevent illness.

Many systematic analyses of rose hips, their antioxidant capacity and potential medicinal use were made recently [1], [4-7], but there is no information on how much of vitamin C and other antioxidants are present in rose hips grown in Slovenia. There are also no available data on how the ripening in late autumn influences those

[1] Biotechnical Faculty, Department of Food Science and Technology, Jamnikarjeva 101, Ljubljana, Slovenia.

parameters and when is the best time to collect the hips to obtain the fruits with the highest levels of antioxidants.

2. Objectives

- to evaluate the total phenolics (TP), antioxidant capacity (AOC) and vitamin C content of rose hips, harvested at ten different locations in Slovenia
- to study the differences in antioxidants of rose hips, matured on the sun and on the shade
- to evaluate the influence of ripening on TP, AOC and vitamin C
- to follow the ratio between oxidised and reduced form of vitamin C during ripening

3. Material and Methods

3.1. Chemicals

Luminol (3-aminophthalhydrazide) and Folin-Ciocalteu reagent were purchased from Fluka, Germany. HRP (horseradish peroxidase) E.C. 1.11.1.7 (269 U mg^{-1}), chlorogenic acid, AA (ascorbic acid), MFK (meta-phosphoric acid), and Tris [2-carboxyethyl] phosphine hydrochloride (TCEP) were from Sigma-Aldrich, Germany. Dimethyl-sulfoxide (DMSO), methanol, HCOOH, H_2SO_4, Na_2CO_3, H_2O_2 and Na_2HPO_4 were from Merck, Germany.

3.2. Collection and extraction of rose hips

Rose hips were collected twice, in September and at the end of October (with 42 days difference), on ten different locations throughout Slovenia (Figure 1). Rose hips on location 7 (Koblarji) were harvested from two different plants, one growing on the sun and the other one on the shade. Samples on location 7 (Ljubljana) were collected four times, in September, in October (after 42 days), in November (after 67 days), and in December (after 94 days).

All the collected hips were within 24 hours after harvesting extracted as follows: 6 g of collected hips (whole hips) was homogenised with 18 g of 2% metaphosphoric acid (in water), filtered through cellulose filters, and centrifuged for 5 min at 14,000× g at room temperature. The supernatants were filtered through CA filters (0.20 µm). Extracts were stored frozen (-20 °C) until analysed.

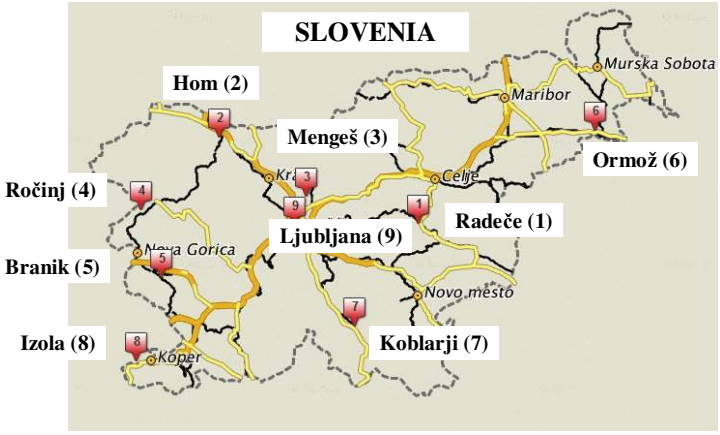

Fig. 1. *Harvesting locations*

3.3. Total phenolics (TP) assay by using Folin-Ciocalteu reagent

Total phenolics (TP) were determined spectrophotometrically using Folin-Ciocalteu (F-C) reagent. To obtain the calibration curve the reaction mixture contained 725 μL of standard solution of chlorogenic acid (3.4 – 58 μmol L^{-1}) and 125 μL of F-C reagent (freshly diluted with water; 1:2). After exactly 5 minutes 125 μL of 20% water solution of Na_2CO_3 was added. Solutions were mixed well and kept in the dark at ambient temperature for 1 hour to complete the reaction. Absorbance at 746 nm was measured by a spectrophotometer (Hewlett- Packard, model HP-8453, USA) against blank sample. To determine TP in herbal extracts, 5 μL of each extract was diluted with 2% MFK to 725 μL and the assay was performed as described above. With all samples assay was conducted in triplicate and the results were averaged. TP of the herbal extracts was expressed as equivalent of chlorogenic acid that gives same signal as the sample.

3.4. Antioxidant capacity (AOC) by chemiluminescence assay

Chemiluminescence was followed by a fluorimeter (Cary Eclipse, Varian, Australia), having the excitation lamp off and using only the photomultiplier at 420 nm.

According to previously optimized chemiluminescent assay [3] the reaction mixture was prepared as follows: 0.188 M luminol stock solution was prepared in DMSO and kept on dark and cold until diluted 1:100 (making 1.88 mM solution) with 0.1 M phosphate buffer (pH 7.4) prior to experiments. Afterwards, 4.5 mL of 1.88 mM luminol solution and 0.5 mL of 80 mM H_2O_2 in phosphate buffer (pH 7.4) were mixed and 2 mL of the so prepared mixture was then put to cuvette, to which 70 μL of phosphate buffer (pH 7.4) and 100 μL of HRP solution (3 U/mL) was added. The chemiluminescence of the mixture was inhibited when 5 μL of the sample (standard solutions of AA or rose hip extracts) was added. The time of inhibition corresponded to the AOC of the sample.

With all samples the assay was conducted in triplicate and the results were averaged. AOC of the herbal extracts was expressed as equivalent of ascorbic acid that gives same signal as the sample.

3.4. Chromatographic analysis of ascorbic acid (AA)

Samples were analysed by HPLC (Marathon-XTautosampler, Knauer isocratic Pump (K-1001), X-Act degassing unit from Jour research, Knauer UV–VIS detector, Wellchrominterface box and PC running EuroChrom 2002 software).

Ascorbic acid (AA) was analysed on a Synergy Hydro-RP 80 (4 μm, 250×4.60 mm, Phenomenex) chromatographic column, equilibrated with 2.5 mM H_2SO_4 in Milli-Q water at a flow rate of 1 mL/min. 20 μL samples were injected on the column. AA, eluted after 4.9 min, was detected at 250 nm.

To determine the total vitamin C in rose hip extracts all dehydroascorbic acid (DHA) was fully reduced with TCEP prior to HPLC analysis. For this purpose 600 μL of 11 mM TCEP in 2% metaphosphoric acid was added to 600 μL of water herbal extracts.

4. Results and Discussions

4.1. Influence of location and harvesting season on total antioxidants

Total phenolics

To evaluate the TP in samples collected at 10 different locations in September and

October, F-C assay was performed. The results on Figure 2 show that there are big differences in TP values of rose hips collected on different locations throughout Slovenia. The highest TP (0.108 mmol/g) at the first harvesting (in September) were determined on location 6 (Ormož) and location 8 (Izola), which are the places with highest average temperature among the selected locations. The lowest level of TP (0.066 mmol/g) was determined in hips harvested at location 4 (Ročinj). The hips harvested on the sunny location at Koblarji contained 55% more TP than hips harvested at same location, but from the plant grown on the shade. In all cases except in the hips harvested at location 7 (Koblarji – sun), the TP levels increased during the ripening. The increase varied from as little as 4% in the case of location 9 (Ljubljana) up to 52% at location 6 (Ormož).

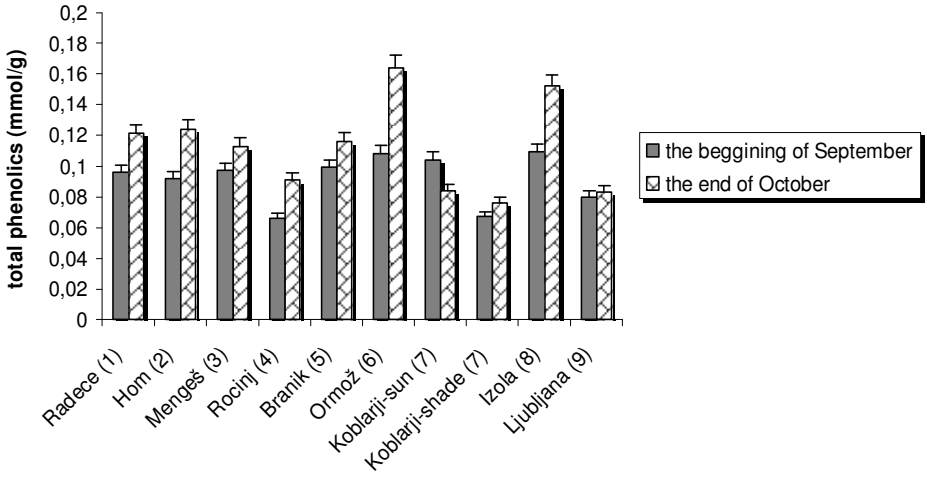

Fig. 2. *Influence of harvesting season on total phenolics. TP is expressed as equivalent of chlorogenic acid.*

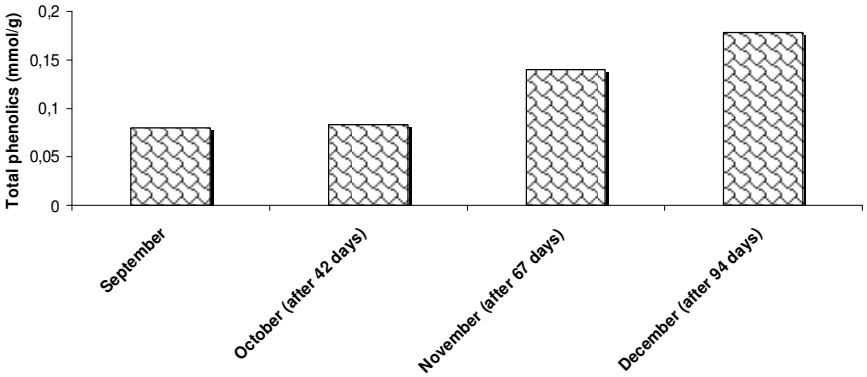

Fig. 3. *Influence of harvesting season on total phenolics. Samples were collected at location 7 (Ljubljana). TP is expressed as equivalent of chlorogenic acid.*

It was additionally found out that TP levels increase also later during the autumn (Figure 3), since the level of TP harvested in December (94 days after first harvesting) at location 9 (Ljubljana) increased by 122% compared to rose hips, harvested in September. The increased TP can be attributed to coloured polyphenols that develop late during ripening and make the hip more intensively coloured, but partially also due to the reduced water content of hips in December compared to the hips collected in September, since all the results are expressed as chlorogenic acid equivalents per gram of fresh hip.

Antioxidant capacity

The chemiluminescence assay for evaluation of AOC was used to compare the levels of antioxidants of the collected samples. The results on Figure 4 show that AOC depend considerable on the location and season of harvesting. In almost all cases (except location 1 – Radeče and location 6 - Ormož) AOC of the rose hips collected at the end of October were higher than AOC of the rose hips harvested in September. The AOC increased up to 154% (location 2 –Hom) compared to the hips collected in September. The increase was in almost all cases more pronounced than previously discussed TP levels. It is also interesting to note that AOC of hips collected at location 6 (Ormož) is the lowest, whereas TP of hips collected on the same location was nearly the highest among the studied locations. It means that the antioxidants profile in hips collected at different locations is considerably different. Similarly to results of TP it can be again noticed that AOC of hips collected from the sunny location at Koblarji is much higher (almost 100%) than AOC of the hips collected at same location in the shade.

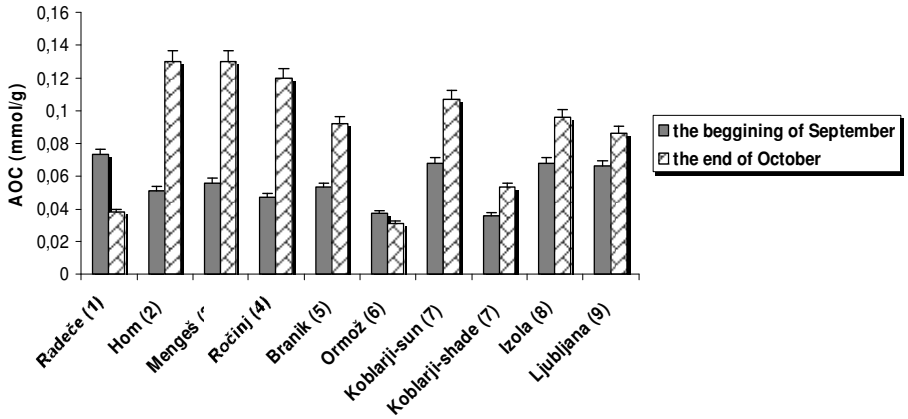

Fig. 4. *Influence of harvesting season on antioxidant capacity (AOC).AOC is expressed as equivalent of ascorbic acid.*

4.3. Influence of location and harvesting season on vitamin C

Vitamin C concentration was in all collected rose hips determined by HPLC/UV-Vis system. It can be seen from the results on Figure 5 that the levels depend both on the location and harvesting

season. But in the contrary to the results of TP and AOC, the amount of vitamin C decreases during the ripening, which is the case of all the samples, except rose hips, collected at location 2 (Hom), which was the poorest in vitamin C concentration at the first collection and it is at the same time the coldest place among the selected locations. The vitamin C concentrations vary from as low as 0.06% to as high as 0.48%, which is eight times more. It was also found out that vitamin C content decreases even more during the late autumn (Figure 6), since the rose hips collected at location 9 (Ljubljana) in December, 94 days after the first collection in September, maintained only 60% of the initial vitamin C.

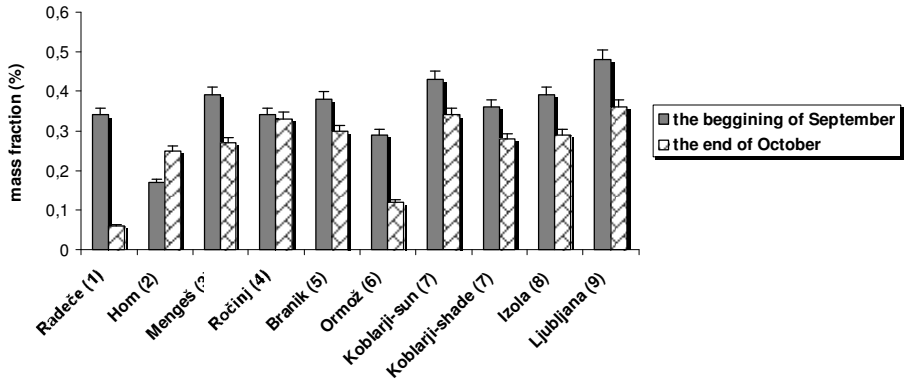

Fig. 5. *Influence of harvesting season on vitamin C content.*

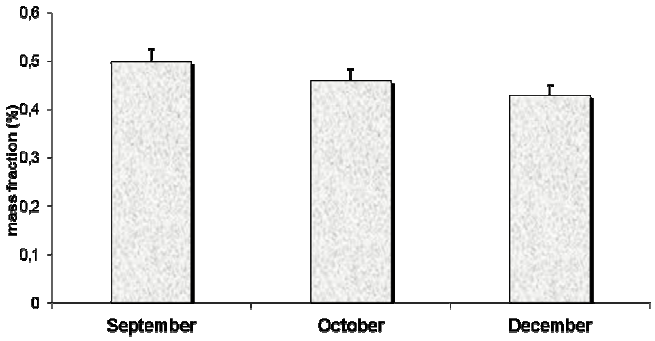

Fig. 6. *Influence of harvesting season on vitamin C. Samples were collected at location 7 (Ljubljana).*

The presence of reduced (AA) and oxidized (DHA) form of vitamin C was also studied in the course of this work. It was found out (Figure 7) that relatively few DHA (below 10%) is present in the rose hips harvested in September. Most of the vitamin C is present in its reduced (more stable) form. In October samples on the other hand much more less-stable DHA is present (Figure 8), in some cases (Ormož) almost the same amount as the reduced form of vitamin C. That coincides with lower levels of overall vitamin C in hips harvested in October and December (Ljubljana).

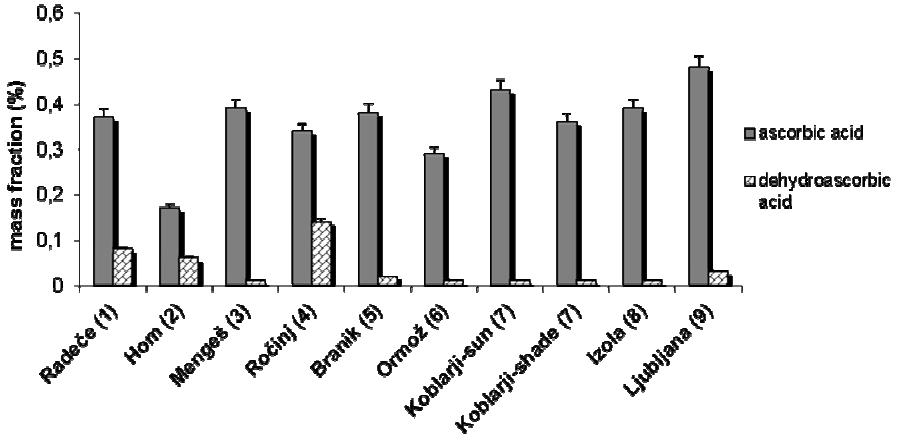

Fig. 7. *Vitamin C (ascorbic acid and dehydroascorbic acid) content of the samples harvested in September.*

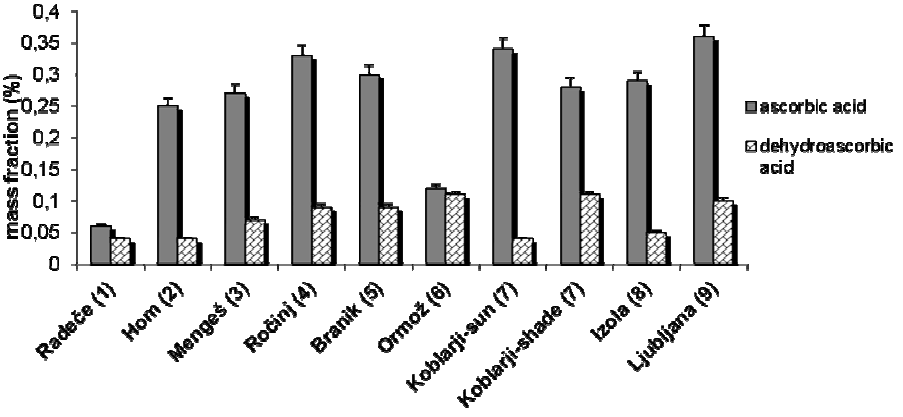

Fig. 8. *Vitamin C (ascorbic acid and dehydroascorbic acid) content of the samples harvested in October.*

5. Conclusions

Total phenolics (TP), antioxidant capacity (AOC) and vitamin C content of rose hips, harvested twice (in September and late October) at ten different locations in Slovenia were determined in the course of this study. The level of TP in fresh rose hips varied between 0.066 mmol/g and 0.164 mmol/g, expressed as chlorogenic acid equivalents. The AOC was between 0.031 mmol/g and 0.13 mmol/g, expressed as AA equivalents and the vitamin C concentration was between 0.06% and 0.48%. The determined values vary regarding the location and the harvesting season. It was found out that during the ripening in late autumn the concentration of vitamin C decreases, while the TP and AOC increase. During the studied period the level of DHA increases, which coincides with the decreased level of AA.

It can be therefore concluded that rose hips have to be collected in early autumn

in order to obtain fruits with higher levels of vitamin C. But at same time one has to be aware that the AOC and TP in that case will be lower due to lower levels of coloured polyphenols that develop only later during the ripening and contribute to higher AOC and TP.

This study was the first systematic study of influence of the environmental factors (namely location, exposure to sun and ripening status) on the levels of antioxidants in rose hips not only in Slovenia, but also elsewhere. In the literature we found some studies regarding antioxidants content in different species of *Rosa spp*., harvested in Turkey [2] and in Canada [9], but none of the two studies was systematically following what is happening with antioxidants during the ripening which on our opinion would have to be important information in order to pick up fruits with the optimal antioxidants content.

Acknowledgements

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