THE CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN SOME FRUITS FROM NATURAL STATE

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Summary
The ornamental plants with edible fruits can play several important functions, ensuring food for the wild animals and embellishing city parks, also these fruits contain high level of bioactive compounds and therefore provide valuable endorsement to the everyday diet. The previous studies of chemical composition of such fruits were fragmentary and superficial. Therefore, the purpose of the study was to analyse the content of major bioactive substances in these fruits.

The experiment was performed at the Laboratory of Division of Organic Food in 2010. The fruits of rowanberry (Sorbus aucuparia L.), flowering quince (Chaenomeles japonica (Thunb.) Lindl. ex Spach), wild rose (Rosa rugosa Thunb.) and berry apple (Malus baccata) were selected for the study. The content of dry matter (by gravimetric method), vitamin C (by colorimetric method), carotenoids and polyphenols (by HPLC) have been determined. The results collected indicate that the fruits of wild rose were characterized by the highest diversity of biologically active compounds among the species examined. At the same time, in these fruits the highest content of vitamin C, lycopene, gallic acid, D-quercetin glucoside, and myricetin has been found compared to the fruits of the other species tested. The highest content of beta-carotene was typical for rowanberry fruits and they contained chlorogenic and cafféic acids as well as quercetin and luteolin which were not detected in the fruits of the other species. Flowering quince and berry apple fruits contained significantly more lutein in comparison with other unusual fruit species examined.

key words: rowanberry, wild rose, berry apple, flowering quince, polyphenols, carotenoids

INTRODUCTION
The species of ornamental plants with edible fruits perform many functions: environmental (ornamental
compounds, acting positively on the human body), and nutritional (one can make various products from the fruits, used as additives in traditional cuisine). Rowanberry Sorbus aucuparia L. is known for its beautiful red fruits. The fruits are used in the treatment of intestinal obstruction, chronic diarrhea, various liver and gallbladder diseases (Wiska-Jeszka et al. 1992). The few qualitative studies indicate that rowanberry fruits contain numerous compounds from the polyphenol group: quercetin, isoquercetin, hyperin, rutin, meratin, kaempferol-O-glycoside, catechin, epicatechin and compounds from the anthocyanin group: cyanidin-3-galactoside, and cyanidin-3,5-diglucoside (Häkkinen & Auriola 1998). Rowanberry fruits contain carotenoids. The largest group of these compounds represent carotenes, which account for 39-50% of all pigments (Goodwin 1956). The cultivated rowanberry fruits contained from 7.25 mg·100 g⁻¹ f.w. to 10.48 mg·100 g⁻¹ f.w. of carotenoids (Kampuss et al. 2009). Rowanberry jam is a wonderful additive to meat dishes. Flowering quince (Chaenomeles japonica L.) is characterized by small and hard fruits of the colour from green to dark yellow depending on a variety. These fruits are delicious and extremely suitable for preserves or as an additive to tea. Flowering quince fruits are rich in fibre, simple sugars as well as a number of compounds from the polyphenol group: catechin, epicatechin, and procyanidins (Wojdylo et al. 2008, Hamauzu et al. 2010).

Rugosa rose (or wild rose) fruits (Rosa rugosa Thunb.) are one of the best sources of vitamin C and caroteneoids. According to Mabellini et al. (2011), a content of vitamin C in wild rose fruits ranged from 843 mg·100 g⁻¹ f.w. to 1230 mg·100 g⁻¹ f.w., while in the case of carotenoids it was from 4.26 mg·100 g⁻¹ f.w. to 4.89 mg·100 g⁻¹ f.w. In addition, the wild rose fruits also contain anthocyanins amounting from 2.8 mg·100 g⁻¹ f.w. to 3.9 mg·100 g⁻¹ f.w. The wild rose fruits represent a great raw material for preserves and jams production. Their weak spasmolytic, choleretic and mildly diuretic effects are used in herbal medicine (Kazaz et al. 2009). Berry apple is characterized by small yellow fruits with a nice red blush. The fruits can be eaten fresh or sugar-candied. The previous studies on the chemical composition of the fruits described are fragmentary and superficial. Therefore, the purpose of the study was a detailed analysis of the content of major bioactive substances in these fruits.

MATERIAL AND METHODS

The experiment was performed at the Laboratory of Division of Organic Food in 2010. The fruits of rowanberry (Sorbus aucuparia L.), flowering quince (Chaenomeles japonica (Thunb.) Lindl. ex Spach), wild rose (Rosa rugosa Thunb.) and berry apple (Malus baccata) were intended for the study. The fruits were harvested from the natural state. All fruits were collected at the same stage of ripeness according to specification described by Ożarowski and Jarosniewski (1987) and Strzelecka and Kowalski (2000). Fruits were selected with respect to uniformity of shape and colour; 1.5 kg of fruits from each
species were collected from 10 trees or bushes in one park and washed. Next the inedible parts of fruits (seeds and peel) were separated from the flesh by means of the rustless scalpel, and only flesh has been next analysed. It was freeze-dried. Dry matter content was determined in fresh flesh without seeds and peel (PN-R-04013:1988). After freeze-drying, the samples were stored in the temp. -80°C to prevent losses in the content of biologically active compounds. Next the samples were grinded in a special lab grinder. In such way the average representative sample has been obtained. Each fruit analysis has been conducted in 10 replications - everyone taken as the weighted portion from the average representative sample of the fruit material.

In the fruits examined, a content of vitamin C was determined according to PN-A-04019:1998, which is presently in force. In the case of presented data only rowanberry fruits gave the colorful extract after the extraction with 2% oxalic acid and here the method of the extraction with xylene and spectrophotometric method have been applied. The rest of fruits (flowering quince, wild rose and berry apple) gave the colourless extracts (the pigments were stopped at the filter paper) and therefore a titration method has been used. Such procedure ensured in the authors’opinion credibility of the obtained results.

Carotenoids were determined by the method described by Helsper et al. (2003) with some modifications (author’s modification 2011). In short, the examined freeze-dried fruits sample was weighed and put into the plastic test tube, having added 0.1% BHT in hexane, magnesium carbonate; the samples were incubated in an ultrasonic bath. Then hexane was added and it was incubated in the bath again. The samples were swirled at the speed of 5,000 rpm. From the test tube 1 ml of supernatant was collected and re-swirled at the speed of 16,000 rpm. The amount of 900 µl of supernatant was taken for HPLC vials and analyzed. To determine carotenoids there was used high performance liquid chromatograph HPLC Shimazu, consisting of two LC-20AD pumps, CM20A system controller, SIL-20AC autosampler, UV/VIS SPD-20AV detector, CTD-20AC oven, and Max-RP 80A column (250 x 4.60 mm). There was selected an isocratic solvent (methanol), flow 1 ml/min. The wavelength used was 450-470 nm. To identify compounds there was used an external standard in the form of lycopene (Sigma Aldrich) and beta-carotene (Fluka) with purity of 99.98%. The phenolic compounds were determined by the method described by Hertog et al. (1992) with own modifications. A weighted amount of freeze-dried fruits sample was put into a plastic test tube, then methanol with ascorbic acid were added, mixed thoroughly by vortex and incubated in an ultrasonic bath. Then the samples were swirled at the speed of 5,000 rpm. From the test tube 1 ml of extract was collected and re-swirled at the speed of 12,000 rpm. The amount of 500 µl of extract was taken for HPLC vials and analyzed. For the analysis of phenolic compounds there was used Synergi Fusion-RP 80i column (250 x 4.60 mm). The gradient flow was applied along
with two mobile phases - acetonitrile/deionized water. Time of the analysis 36 minutes, flow ml/min., wavelength 250-370 nm. Polyphenols were identified based on Fluka and Sigma Aldrich external standards with purity of 99.5%.

The results were based on 10 replications of samples (see above) and the means and standard deviations were calculated. For statistical calculations two-way analysis of variance with the use of the Tukey’s test (α=0.05) has been selected. Lack of statistically significant differences between the examined groups was determined with the same letters.

RESULTS

The results of the content of dry matter, vitamin C and carotenoids are presented in Table 1. Every result is informing about the average level of the particular bioactive compound (e.g. vitamin C) in the fruits from 10 trees or bushes of the studied plant. The fruits of the species examined differed significantly from each other in terms of dry matter content. The highest level of dry matter was found in the fruits of wild rose, while the lowest amount was typical for the fruits of flowering quince. Wild rose fruits were the most abundant in vitamin C. Berry apple fruits, however, had significantly the lowest content of vitamin C (Table 1). A significantly higher level of beta-carotene was confirmed for the rowanberry fruits in comparison with the other ones. The lowest amount of the carotenoid was found in flowering quince and berry apple fruits. Wild rose fruits were characterized by a significantly higher content of lycopene, while this pigment was not present in the fruits of rowanberry. Berry apple fruits contained significantly more lutein compared with the fruits of the other species tested. The lowest quantity of lutein was found in the fruits of wild rose, and the differences were statistically significant (Table 1).

Table 1. The content of dry matter (g·100 g⁻¹), vitamin C (mg·100 g⁻¹ f.w.) and carotenoids (µg·1 g⁻¹ f.w.) in selected fruits from natural stage. Mean value ± standard deviation

<table>
<thead>
<tr>
<th>Species</th>
<th>dry matter</th>
<th>vitamin C</th>
<th>beta-carotene</th>
<th>lycopene</th>
<th>lutein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rowanberry (Sorbus aucuparia L.)</td>
<td>30.7±0.7b**</td>
<td>131.2±0.4c</td>
<td>99.0±2.4c</td>
<td>n.d.***</td>
<td>7.5±0.2b</td>
</tr>
<tr>
<td>Flowering quince (Chaenomeles japonica)</td>
<td>12.8±0.2a</td>
<td>62.7±10.5b</td>
<td>1.7±0.1a</td>
<td>20.5±0.6b</td>
<td>39.7±5.2c</td>
</tr>
<tr>
<td>Wild rose (Rosa rugosa Thunb.)</td>
<td>33.6±1.1b</td>
<td>157.5±4.4d</td>
<td>43.6±4.8b</td>
<td>425.1±10.4e</td>
<td>3.1±0.1a</td>
</tr>
<tr>
<td>Berry apple (Malus baccata L.)</td>
<td>17.3±1.9a</td>
<td>42.0±3.0a</td>
<td>1.7±0.2a</td>
<td>10.5±1.1a</td>
<td>86.8±24.6d</td>
</tr>
</tbody>
</table>

p-value species <0.0001* <0.0001 <0.0001 <0.0001 0.0001
* statistical significant on the level of probability α=0.05; ** means in a column followed by the same letter are not significantly different at the 5% level of probability (α=0.05);
*** n.d. compound not detected or trace finger
The content of total phenolic acids and total flavonoids is shown in Fig. 1. The collected results indicate that the highest level of total phenolic acids contained the fruits of wild rose, while berry apple fruits did not contain phenolic acids at all. The highest level of total flavonoids was found in rowanberry fruits, while the lowest quantity was confirmed for the fruits of berry apple, and the differences were statistically significant (Fig. 1.).

![Fig.1. The content of total phenolic acids and total flavonoids in fruits from natural stage](image)

Fig. 1. The content of total phenolic acids and total flavonoids in fruits from natural stage

The fruits studied differed not only in quantitative but also qualitative composition in terms of the content of these antioxidant compounds. Two phenolic acids were present in the fruits of rowanberry: chlorogenic and caffeic acids. Flowering quince fruits contained only p-coumaric acid, while gallic acid was found in wild rose fruits (Table 2). Wild rose fruits – as the only – contained D-quercetin glucoside and significantly more D-kaempferol glucoside and myricetin in comparison with the other fruits examined. 3-quercetin rutinoside was found only in flowering quince fruits. Quercetin and luteolin occurred only in the fruits of rowanberry, while they were not found in the other fruits (Table 3).
Table 2. The content of phenolic acids in selected fruits from natural stage. Mean value ± standard deviation

<table>
<thead>
<tr>
<th>Species</th>
<th>phenolic acids (mg·100 g⁻¹ f.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gallic</td>
</tr>
<tr>
<td>Rowanberry (Sorbus aucuparia L.)</td>
<td>n.d.**</td>
</tr>
<tr>
<td>Flowering quince (Chaenomeles japonica)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Wild rose (Rosa rugosa Thunb.)</td>
<td>92.4±26.3</td>
</tr>
</tbody>
</table>

p-value

| species | <0.0001* | <0.0001 | <0.0001 | <0.0001 |

* statistical significant on the level of probability α=0.05
** n.d. compound not detected or trace finger

Table 3. The content of flavonoids in selected fruits from natural stage. Mean value ± standard deviation

<table>
<thead>
<tr>
<th>Species</th>
<th>flavonoids (mg·100 g⁻¹ f.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D-quercetin glucoside</td>
</tr>
<tr>
<td>Rowanberry (Sorbus aucuparia L.)</td>
<td>n.d.***</td>
</tr>
<tr>
<td>Flowering quince (Chaenomeles japonica)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Wild rose (Rosa rugosa Thunb.)</td>
<td>13.7±0.8</td>
</tr>
<tr>
<td>Berry apple (Malus baccata L.)</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

p-value

| species | <0.0001* | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

* statistical significant on the level of probability α=0.05
** means in a column followed by the same letter are not significantly different at the 5% level of probability (α=0.05)
*** n.d. compound not detected or trace finger

DISCUSSION

The dry matter content was significantly dependent on the fruit species examined. Rowanberry fruits contained up to 30.7 g·100 g⁻¹ f.w. of dry matter. Kampuss et al. (2009) received lower results, which fall within the range of 6-18 g·100 g⁻¹ f.w. for ten different varieties of rowanberry. The high dry matter content was also found in the fruits of wild rose (33.6 g·100 g⁻¹ f.w.). However, Mabellini et al. (2011) presented
much lower values of 12.4–20.8 g·100 g⁻¹ f.w. Berry apple fruits contained 17.3 g·100 g⁻¹ f.w. of dry matter (Table 1). Similar results were confirmed only in one paper. In the case of the fruits of berry apple, Padewsk (2008) received the dry matter content in the amount of 17.2 g·100 g⁻¹ f.w. Rowanberry is the species rich in vitamin C. The content of vitamin C in wild rose and rowanberry fruits is similar to blackcurrant fruits, so these wild fruits could be a good source of vitamin C in diet. According to the study presented, the vitamin C content in rowanberry fruits was equal to 131.3 mg·100 g⁻¹ f.w. (Table 1). These results are similar to the ones presented by Piir et al. (2003), who indicated the range of vitamin C content in rowanberry fruits of 12-86 mg·100 g⁻¹ f.w., which was higher than the result provided by Kampuss et al. 2009 (10-51 mg·100 g⁻¹ f.w.). Most vitamin C was found in wild rose fruits - 157.5 mg·100 g⁻¹ f.w. (Table 1). These results are significantly lower than the ones presented by Nojvan et al. (2008), Kazaz et al. (2009) and Mabellini et al. (2011). According to the papers by these authors, the vitamin C content in wild rose fruits ranged from 411 mg·100 g⁻¹ f.w. up to 2200 mg·100 g⁻¹ f.w. Flowering quince fruits contained significantly the lowest level of vitamin C (Table 1). A higher content of vitamin C in flowering quince fruits was obtained by Ros et al. (2004) and Tang et al. (2009), and it ranged from 30 mg·100 g⁻¹ f.w. to 64 mg·100 g⁻¹ f.w. The vitamin C content in berry apple fruits was one of the lowest among the species studied (Table 1). The vitamin C content in these fruits was similar to that given by Padewska (2008). In the research presented, the content of total carotenoids in rowanberry fruits was 106.5 mg·100 g⁻¹ f.w. (Table 1), and beta-carotene alone accounted for 93%. Kampuss et al. (2009) received a significantly lower content of total carotenoids, as it was the range of 7.2-10.5 mg·100 g⁻¹ f.w. However, Piir et al. (2003) reported that the beta-carotene content in the fruits of rowanberry amounted to 12-22 mg·100 g⁻¹ f.w. The species with the highest abundance of carotenoids was wild rose. In the studies presented, the total carotenoid content amounted up to 417.9 mg·100 g⁻¹ f.w., out of which lycopene accounted for 91% of all carotenoids. These results are consistent with the ones presented by Mabellini et al. (2011), who received the total carotenoid content in the fruits of wild rose at the level of 426-489 mg·100 g⁻¹ f.w. In the studies presented, the beta-carotene content in wild rose fruits was 43.6 mg·100 g⁻¹ f.w. (Table 1). This result was significantly higher than the one presented by Kazaz et al. (2009), where the beta-carotene content in wild rose fruits amounted to 0.2-3.3 mg·100 g⁻¹ f.w., which was much lower (217-268 mg·100 g⁻¹ f.w.) than the result indicated by Ghazghazi et al. (2010). It may point out a huge diversity of the pigment content, depending on the source of the fruits as well as natural conditions in which shrubs were grown. The fruits of berry apple contained 86.7 µg·g⁻¹ of lutein, 10.5 µg·g⁻¹ lycopene and 1.7 µg·g⁻¹ beta-carotene. The results are higher from that presented by Setiawan et al. (2001), who reported that indonesian apple contained 1.1 µg·g⁻¹ of cryptoxanthin, 2.1
µg·g⁻¹ of lycopene and 0.7 µg·g⁻¹ of beta-carotene. In the study presented, the content of polyphenolic compounds in the fruits of rowanberry was 176.7 mg·100 g⁻¹ f.w. (Fig. 1), out of which chlorogenic acid accounted for 22.3%. A higher content of these compounds in rowanberry fruits was provided by Hukkanen et al. (2006), and it was the range of 550-1014 mg·100 g⁻¹ f.w., while chlorogenic acid accounted only for 10.5% of all phenolic compounds in rowanberry fruits. The fruits of wild rose contained 176.1 mg·100 g⁻¹ f.w. of polyphenolic compounds (Fig. 1). The results are similar to those presented by Ercisli (2007), who received the total polyphenol content in wild rose fruits at the level of 96 mg·100 g⁻¹ f.w., which was significantly higher than the result indicated by Ghazghazi et al. (2010) - only 5.4-8.4 mg·100 g⁻¹ f.w. of polyphenolic compounds. The fruits of flowering quince were characterized by a relatively low content of total phenolic compounds (Fig. 1), and it was 19.3 mg·100 g⁻¹ f.w. The results are similar to those presented by Tang et al. (2009), who received the total phenolic content in flowering quince fruits of 50 mg·100 g⁻¹ f.w. In the paper presented, berry apple fruits contained 15.3 mg·100 g⁻¹ f.w. of total flavonoids (Fig. 1). It is consistent with the studies by Padewska (2008), who reported that the fruits of berry apple contained 6.7 mg·100 g⁻¹ f.w. of these compounds. The species of genus Malus are rich in such phenolic compounds as: chlorogenic acid and quercetin. According to the results of Awad et al. (2000), apples contained wide range of chlorogenic acid 0.4-2.0 mg·100 g⁻¹ f.w. Also results obtained by Boyer and Liu (2004) showed that apples contained 13.2 mg·100 g⁻¹ f.w. of quercetin glycoside and 9.0 mg·100 g⁻¹ f.w. of chlorogenic acid. In the presented paper chlorogenic acid and quercetin were not detected in berry apple fruits.

CONCLUSIONS

1. Wild rose fruits had a significantly higher content of dry matter, vitamin C, total phenolic acids, gallic acid, D-quercetin glucoside, D-kaempferol glucoside, and myricetin compared with the fruits of other species examined.
2. Rowanberry fruits contained significantly more beta-carotene, total flavonoids, chlorogenic acid, caffeic acid, quercetin and luteolin than the fruits of other species examined.
3. Berry apple fruits contained significantly more lutein compared with the fruits of other species examined.
4. Rutin and p-coumaric acid were only found in flowering quince fruits. These compounds were not confirmed in the fruits of other species examined.

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