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BIOCHEMICAL COMPOSITION AND ANTIRADICAL ACTIVITY OF ROWANBERRY (*SORBUS L.*) CULTIVARS AND HYBRIDS WITH DIFFERENT *ROSACEAE* L. CULTIVARS

PĪLĀDŽU (*SORBUS* L.) ŠĶIRŅU UN TO HIBRĪDU AR CITIEM *ROSACEAE* L. AUGĻAUGIEM ANTIOKSIDATĪVĀ AKTIVITĀTE UN BIOĶĪMISKAIS SASTĀVS

Kaspars Kampuss[1], Solvita Kampuse[2], Elga Berņa[2], Zanda Krūma[2], Inta Krasnova[3], Inese Drudze[4]

[1] Faculty of Agriculture, Latvia University of Agriculture (LLU), Liela street 2, Jelgava, Latvia
[2]Faculty of Food Technology, LLU, e-mail: skampuse@inbox.lv
[3] The Latvia State Fruit Growing Institute, Dobele, Latvia
[4] Pure Horticultural Research Centre, Abavas 2, Pure, LV-3124, Latvia

Abstract

Rowanberry (*Sorbus aucuparia* L.) is a common yellowish, wild berry that grows in the Northern part of Europe. Rowan has been described as an important source of flavonoids and their antioxidant activity affects reactive oxygen species and lipid peroxidation. The aim of this study was to evaluate the biochemical composition of 8 rowanberry cultivar fruits and the fruits of their hybrids with *Rosaceae* L. cultivars and to establish the correlation of their biochemical composition with antiradical activity. The experiments were done at the Faculty of Food Technology, Latvia University of Agriculture (LLU) and in the Latvia State Fruit Growing institute, Dobele. The content of ascorbic acid, the total phenols, anthocyanins, carotenoids, soluble solids, titrable acids, tannins and the antiradical activity of fresh and frozen rowanberry×hawthorn 'Granatnaya', rowanberry×chokeberry 'Likiornaya', rowanberry *(Sorbus aucuparia)* 'Rosina', rowanberry *(Sorbus aucuparia)* 'Zholtaya', wild rowanberry *(Sorbus aucuparia)*, rowanberry×pear 'Alaya Krupnaya', rowanberry *(Sorbus aucuparia)* 'Rosina Variegata', rowanberry *(Sorbus aucuparia)* 'Krasnaya Krupnaya' were analysed. There were no significant differences between the chemical composition of fresh and frozen rowanberry samples. The highest content of ascorbic acid was in fruits of the rowanberry 'Rosina', 'Rosina Variegata', 'Krasnaya Krupnaya', and 'Zholtaya' (49 – 53 mg 100 g^{-1}). The highest content of carotenoids were detected in rowanberry×hawthorn 'Granatnaya' (13.04 mg 100 g^{-1}), but the highest phenol content was detected in the rowanberry and chokeberry hybrid 'Likiornaya' (484.9 mg 100 g^{-1}). 'Likiornaya' showed also the highest antiradical activity (11.2 g of berries per 1g of DPPH radical).

Kopsavilkums

Pīlādži (*Sorbus aucuparia* L.) ir izplatīts savaļas augļaugs, kas aug Eiropas ziemeļu daļā. Pīlādži ir raksturoti kā nozīmīgs flavonoīdu avots un to antioksidatīvā aktivitāte ietekmē reaktīvo skābekli un lipīdu peroksidāciju. Pētījuma mērķis bija izvērtēt bioķīmisko sastāvu 8 pīlādžu šķirņu un to

hibrīdu ar citiem *Rosaceae* L. augļaugiem, noteikt korelāciju starp to antioksidatīvo aktivitāti un bioķīmisko sastāvu.
Eksperimenti tika veikti Latvijas Lauksaimniecības universitātes Pārtikas tehnoloģijas fakultātē un Latvijas Valsts Augļkopības institūtā 2008. gadā. Pīlādžu šķirņu un hibrīdu paraugi tika ievākti no Pūres Dārzkopības izmēģinājumu stacijas kolekcijas. Analizēts tika svaigu un saldētu pīlādža × vilkābeles 'Granatnaya', pīlādža × aronijas 'Likiornaya', pīlādža *(Sorbus aucuparia)* 'Rosina', pīlādža *(Sorbus aucuparia)* 'Zholtaya', savaļas pīlādža *(Sorbus aucuparia)*, pīlādža × bumbiera 'Alaya Krupnaya', pīlādža *(Sorbus aucuparia)* 'Rosina Variegata', pīlādža *(Sorbus aucuparia)* 'Krasnaya Krupnaya' paraugu askorbīnskābes, kopējo fenolu, karotinoīdu, šķīstošās sausnas, miecvielu saturs un antioksidatīvā aktivitāte.
Starp svaigiem un saldētiem pīlādžu un to hibrīdu paraugiem netika konstatētas būtiskas atšķirības. Augstākais askorbīnskābes saturs bija 'Rosina', 'Rosina Variegata', 'Krasnaya Krupnaya', un 'Zholtaya' paraugiem (49 – 53 mg 100 g^{-1}). Augstākais kopējo karotinoīdu saturs bija pīlādža × vilkābeles 'Granatnaya' augļiem (13.04 mg 100 g^{-1}), bet augstākais kopējo fenolu saturs bija pīlādža un aronijas hibrīdam 'Likiornaya' (484.9 mg 100 g^{-1}). 'Likiornaya' uzrādīja arī augstāko antioksidatīvo aktivitāti (11.2 g ogu uz 1 g of DFPH radikāļa).

Key words: ascorbic acid, phenols, antiradical activity (DPPH), frozen, fresh

Introduction

Rowanberry *(Sorbus aucuparia* L.*)* is a common yellowish, wild berry that grows in the northern part of Europe. They have been described as an important source of flavonoids and their antioxidant activity affects reactive oxygen species and lipid peroxidation (Gil-Izquierdo and Mellenthin, 2001).

The fruits of rowan (*Sorbus aucuparia* L.*)* have been traditionally used for jellies and jams, but their wider use as food ingredients has been less popular because of their bitter taste. The first sweet rowanberry clones were selected in the Sudety mountain area, in the current Czech Republic area in the 19th century. A breeding program for sweet rowanberries was started by Michurin in Russia at the beginning of 20th century, resulting in interesting hybrids of the rowanberry (*Sorbus aucuparia* L.*)* with the *Aronia*, *Malus*, *Mespilus*, or *Pyrus* species. Sweet rowanberries have been bred particularly for northern conditions and have shown excellent winter-hardiness in Russia and Finland. The taste of these berries is less astringent than that of wild rowanberries, and the berries are often larger. The total phenolic content can vary greatly among the sweet rowanberry cultivars ranging from 550 – 1014 mg 100 g^{-1} of fresh weight of berries. A high correlation between the antioxidant capacity and phenolic contents of sweet rowanberries was established (Hukkanen *et al.*, 2006). Including different types of berries other Finnish authors found that the phenolic composition data showed no remarkable correlation between antioxidant activity and total phenolics. A statistically significant correlation was observed between flavonol content and antioxidant activity, and between hydroxycinnamic acid and antioxidant activity (Kähkönen *et al.*, 2001).

Rowanberries contain also carotenoids, vitamin E, and vitamin C, which might also contribute to their antioxidant capacity. According to Piir and Niiberg (2003) carotenoid levels in sweet rowanberries are as high as those in carrots, and levels of vitamin C are close to those of strawberries, varying from 12-21 mg 100 g^{-1} ('Granatnaya') to 86 mg 100 g^{-1} ('Zholtaya') (Piir and Niiberg, 2003; Häkkinen *et al.*, 1999). Also higher vitamin C content is found in the rowanberry varieties (*S. aucuparia*) than in the hybrid cultivars.

The Pure Horticultural Research Centre has a large collection with sweet rowanberry cultivars and their hybrids with *Rosaceae* L. genus cultivars collected from Russia and other countries. But there is still little information about the biochemical composition and the nutritional value of the fruits of these cultivars.

Therefore the aim of this study was to evaluate the biochemical composition and nutritional value of 8 rowanberry cultivars and hybrids with other *Rosaceae* L. genus cultivars and to find correlations between of the biochemical composition and the antiradical activity.

Materials and methods

The experiments were done at the Faculty of Food Technology, Latvia University of Agriculture (LLU), Jelgava, and at the Latvia State Fruit Growing Institute, Dobele. The samples of rowan cultivars and their hybrids were collected from the Pure Horticultural Research Centre collection of genetic resources.

The samples were analyzed fresh and after freezing. After harvesting the rowanberries were sorted, frozen in a freezer PORKKA BF 710 at the temperature of -25±2 °C, then packaged and stored for one month in the freezer chamber VTK 201 U at a temperature of -20±2 °C.

Contents of titratable acids, soluble solids, ascorbic acid, total phenols, carotenoids, and the antiroxidant activity (DPPH) of the fresh and frozen fruits of wild rowanberry (*Sorbus aucuparia*), rowanberry×hawthorn 'Granatnaya', rowanberry×chokeberry 'Likiornaya', rowanberry (*Sorbus aucuparia*) 'Rosina', rowanberry (*Sorbus aucuparia*) 'Zholtaya', rowanberry×pear 'Alaya Krupnaya', rowanberry (*Sorbus aucuparia*) 'Rosina Variegata', and rowanberry (*Sorbus aucuparia*) 'Krasnaya Krupnaya' were analysed.

The content of the ascorbic acid was determined by titration with a 0.05-M iodine solution (Moor *et al.*, 2005). 25 g of berries were doused with 100 ml of 6 % solution of oxalic acid and homogenized for 1 minute. Then the sample was filtered. 2 ml of 1 % solution of starch was added to10 ml of filtrate and the filtrate was titrated until change of colour, which does not disappear during 30 seconds. The content of ascorbic acid mg per 100 g of berries was calculated from the following equation [1]:

$$C = 400 \cdot \frac{V_{sample}}{V_{s\tan dard}}, \qquad [1]$$

where V_{sample} – volume of the iodine solution titrated in a sample, ml;
$V_{standard}$ – volume of the iodine solution titrated in a standard solution, ml.

The total titrable acids were determined by titration with 0.1 N NaOH (ISO 750:1998) in fresh and frozen berries.

The contents of *soluble solids* were determined by a refractometer (ISO 2173:2003) in fresh and frozen berries.

The total phenol content was determined by the photometric method with a Folin-Ciocalteau reagent (Singleton *et al.*, 1999). For analyses of phenols the Folin-Ciocalteau reagent and 4 ml 7.5% sodium carbonate was used. After 30 minutes the samples were analyzed with a spectrophotometer at the wave length of 765 nm. As a control solution 1 ml water with 5 ml Folin-Ciocalteau reagent and a 4 ml 7.5 % sodium carbonate solution was used. The content of phenols is calculated from formula [2]:

$$X = \frac{C}{a \cdot 10}, \qquad [2]$$

where C – content of phenols, mg 100 g^{-1};
a – the amount of analyzed sample, g.

The results of all analyses were recalculated to 100 g of dry weight.

Carotenoids were analyzed by the spectrophotometric method at a wave length of 440 nm (Ермаков , 1987). One to two grams of homogenized berries were placed in a 100 ml conic retort and 20 ml 96 % ethanol was added. The sample was stirred by a magnetic stirrer for 15 min then 25 ml petrol ether was added and stirring was continued for one more hour. After 3 – 4 hours when both layers were completely separated, the top (yellow) layer was used for the further detection of carotenoids at a wave length of 440 nm. The carotene equivalent (KE) was found, using a graduation curve with $K_2Cr_2O_7$. The content of carotenoids (mg 100 g^{-1}) was calculated by equation 4:

$$X = \frac{0.208 \cdot 25 \cdot 100 \cdot KE}{36 \cdot a}, \qquad [4]$$

where 0,208 and 36 coefficients for the relationship between $K_2Cr_2O_7$ and carotenoids;

25 – dilution coefficient;
KE – carotene equivalent from the graduation curve;
a – sample weight, g.

The antiradical activity (ARA) of frozen berries was analyzed by the spectrophotometric method with the N,N-diphenil-N'-picrilhydrazil (DPPH) reagent at a wave length of 517 nm (Milauskas *et al.*, 2004). 50 ml of ethanol was added to 10 g of the homogenized sample; the glasses were closed by Parafilm and stirred for 2 hours for extraction. After 2 hours top clear layer was decanted, 50 ml ethanol added to the sample and the extraction was repeated for 2 hours. 2.9 ml $1\cdot10^{-4}$M DPPH was filled in the cuvette and 100 µl of extract added. The sample was stirred and placed in the dark for 30 min. Then the absorption was measured at 517 nm (spectrophotomter UV – 1650 PC). ARA was calculated by equation 5:

$$ARA = \frac{A_{DFPH} - A_{sample}}{A_{DFPH}} \cdot 100\%, \qquad [5]$$

where: A_{DPPH} – absorption of DPPH reactive;
A_{sample}- absorption of DPPH after addition of fruit extract.

Tannins. The total content of tannins was detected using the traditional method by titration with 0.1 n $KMnO_4$ (Шмыд, 1960). The content of tannins (x) was calculated from formula 6:

$$X = \frac{(v_1 - v_2) \cdot 0.04157 \cdot 100}{a}, \qquad [6]$$

where v_1- the amount of 0.1 n $KMnO_4$ used in the first titration, ml;
v_2 – the amount of 0.1 n $KMnO_4$ used in the second titration, ml;
a – the amount of 0.1 n $KMnO_4$ used for oxidizing of 10 ml of 0.1 oxalic acid, ml.

Results and Discussion

The evaluated components of chemical composition did not significantly (p=0.13-0.59) differ between the fresh and frozen samples that demonstarte the possibility of frozen rowanberry usage similar to the fresh ones.

The ascorbic acid content of rowanberry cultivars and their hybrids is similar to many other fruit species and did not exceed 53 mg 100 g^{-1}, which is similar to that mentioned in literature (Gil-Izquierdo Mellenthin., 2001). The highest content of ascorbic acid was detected in the rowanberry 'Rosina', 'Rosina Variegata', 'Krasnaya Krupnaya', and 'Zholtaya' (Figure 1). The wild rowanberry and hybrids with different *Rosaceae* L. genus cultivars had the significantly lower content of vitamin C.

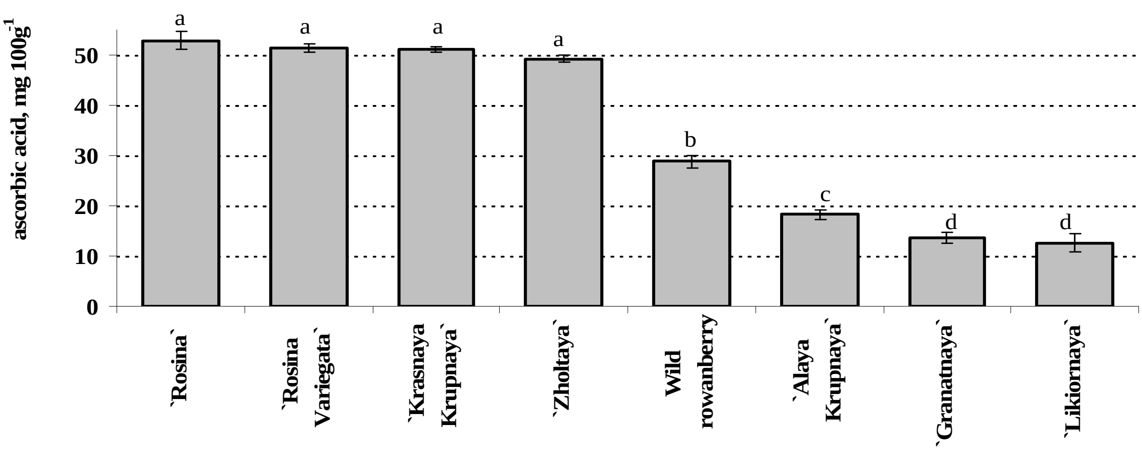

Figure 1. Ascorbic acid content in rowanberries and their hybrids.

**Values, marked with the same letter, are not significantly different at p=0.05*

The titrable acids content of the evaluated rowanberry cultivars and the hybrids was 1.6 – 2.8 % (Table 1). The highest acidity was found in the wild rowanberry, but the lowest titrable acids content were in the hybrids rowanberry×hawthorn 'Granatnaya', and rowanberry×chokeberry 'Likiornaya'.

Table 1. Biochemical composition of rowanberries and their hybrids

Cultivar	Titrable acids, %	Carotenoids, mg/100g	Tannins, %
Rowanberry×pear 'Alaya Krupnaya'	2,61 ± 0,10 [ab]	9,24 ± 0,16 [c]	0,33 ± 0,02 [b]
Rowanberry×hawthorn 'Granatnaya'	1,73 ± 0,04 [d]	13,04 ± 0,68 [a]	0,37 ± 0,01 [ab]
Rowanberry (*Sorbus aucuparia*) 'Krasnaya Krupnaya'	2,65 ± 0,12 [ab]	7,25 ± 0,24 [d]	0,13 ± 0,02 [c]
Rowanberry×chokeberry 'Likiornaya'	1,62 ± 0,02 [d]	10,48 ± 0,05 [b]	0,39 ± 0,02 [ab]
Rowanberry 'Rosina Variegata'	2,67 ± 0,02 [ab]	8,08 ± 0,14 [d]	-
Rowanberry (*Sorbus aucuparia*) 'Rosina'	2,48 ± 0,09 [b]	10,04 ± 0,27 [bc]	0,38 ± 0,00 [ab]
Wild rowanberry (*Sorbus aucuparia*)	2,80 ± 0,03 [a]	9,52 ± 0,31 [bc]	0,42 ± 0,02 [a]
Rowanberry (*Sorbus aucuparia*) 'Zholtaya'	2,09 ± 0,03 [c]	9,53 ± 0,36 [bc]	0,32 ± 0,04 [b]

The soluble solids content in rowanberries varied between 11 and 18 % (Figure 2). An interesting fact was that the highest soluble solid content was in the wild rowanberry (*Sorbus aucuparia*) (18.0 %, in average). All other rowanberry cultivars and hybrids had a significantly lower soluble solid content than the wild rowanberry and did not reach even 16 % though in literature the content of soluble solids in fruits of cultivars 'Rosina' and 'Zholtaya' were mentioned as being above 19 % (Hukkanen *et al.*, 2006). This means that the soluble solids of rowanberries can greatly fluctuate in different regions and during different years.

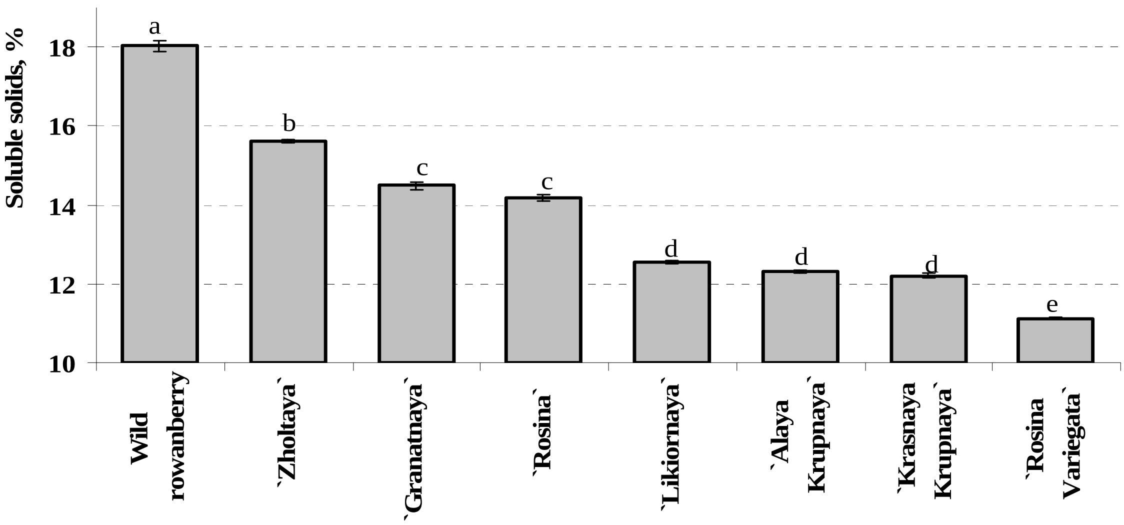

Figure 2. Content of soluble solids in rowanberries and their hybrids.

**Values, marked with the same letter, are not significantly different at p=0.05*

The total phenol content of rowanberries and their hybrids differed between 162 and 485 mg $100g^{-1}$ of fresh weight (Figure 3). It is lower than reported in literature (Hukkanen *et al.*, 2006). The highest phenol content was detected in the rowanberry and the chokeberry hybrid 'Likiornaya' (in average 484.9 mg 100 g^{-1}).

The differences in the *total carotenoid* content of eight rowanberry cultivars and hybrids were between 7 and 13 mg 100 g^{-1} of fresh berry weight (Table 1). The rowanberry and hawthorn hybrid 'Granatnaya' had the highest carotenoid content (13.04 mg 100 g^{-1}, in average).

The total tannin content of the evaluated samples was between 0.3 and 0.4 %, and only rowanberry cultivar 'Krasnaya Krupnaya' had a significantly lower content of total tannins (0.13 % on average) (Table 1). The wild rowanberry had the highest content of tannins (0.42 % in average) which explains the most astringent flavour of these berries compared to other rowanberry cultivars and hybrids.

The DPPH radical scavenging activity of the evaluated samples ranged from 2.5 to 11.2 g of berries per g of DPPH radical (Figure 3). The highest antiradical activity (DPPH) was found in the rowanberry and chokeberry hybrid 'Likiornaya' (11.2 g of berries per g of DPPH radical).

There was a significant (p=0.01) correlation between the antiradical activity and total phenolic content (r=0.960) (Figure 3).

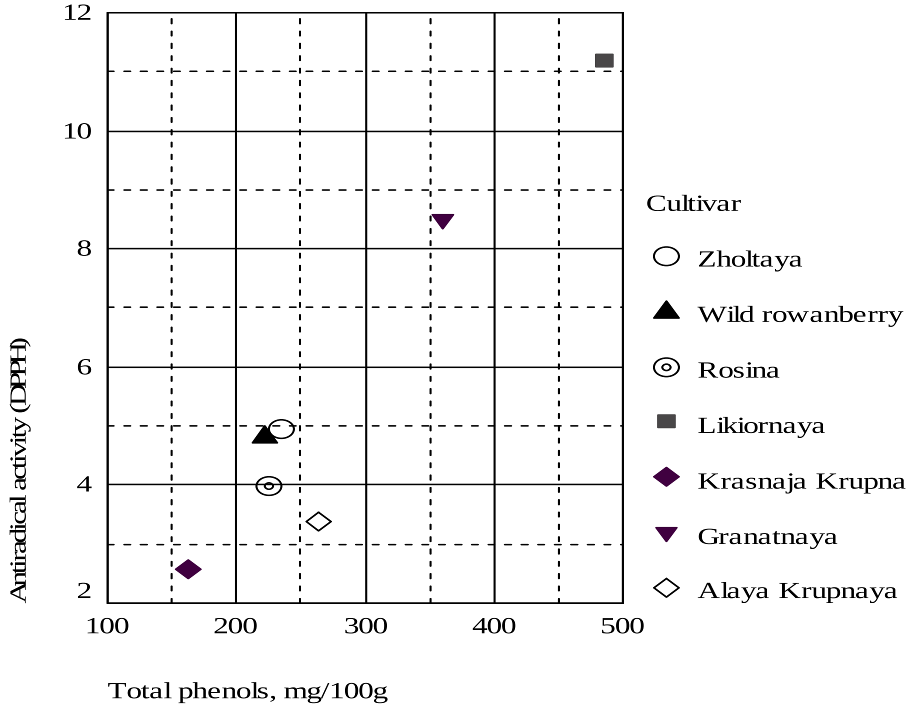

Figure 3. Pearson correlation plot between the antiradical activity (DPPH) and total phenolics of rowanberries and their hybrids.

The correlation between antiradical activity and phenolic content was found also in other investigations with berries (Hukkanen *et al.*, 2006; Gil *et.al.*, 2002). There was no statistically significant correlation between vitamin C content and antiradical capacity and also between carotenoids and antiradical capacity although these bioactive compounds are mentioned as strong antioxidants (Puupponen-Pimiä *et al.*, 2001; Sergio *et al.*, 1999; Sies and Stahl, 1995). This could be explained by the similar amounts of vitamin C and carotenoids in several rowanberry cultivars and hybrids while the phenolic content was different for the three better cultivars. It was also found that a significant negative correlation exists between the antiradical activity and the titrable acid content (p=0.008, r=-0.886). It is difficult to find any explanation for this correlation and it seems that it is specific only to the evaluated rowanberry cultivars.

Conclusions

There were no signifficant diferences between the chemical composition of the fresh and frozen rowanberry samples.

The highest content of ascorbic acid was found in rowanberries ‘Rosina’, 'Rosina Variegata', 'Krasnaya Krupnaya', and 'Zholtaya' (49 – 53 mg 100 g^{-1}). The highest content of carotenoids was detected in the rowanberry×hawthorn 'Granatnaya‘ (13.04 mg 100 g^{-1}), but the highest phenol content was detected in the rowanberry and chokeberry hybrid 'Likiornaya' (484.9 mg 100 g^{-1}). 'Likiornaya' also had the highest antiradical activity (11.2 g of berries per 1g of DPPH radical).

There was a significant correlation established only between the antioxidant activity and the total phenolic content (r=0.886).

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RESEARCH ON THE MINERAL COMPOSITION OF AMERICAN CRANBERRIES AND WILD CRANBERRIES IN LATVIA
AMERIKAS LIELOGU UN SAVVAĻAS DZĒRVEŅU OGU MINERĀLĀ SASTĀVA SALĪDZINOŠS IZVĒRTĒJUMS

Andis Karlsons, Anita Osvalde, Vilnis Nollendorfs

Institute of Biology, University of Latvia, Miera Street 3, Salaspils LV-2169, Latvia,
e-mail: augi@email.lubi.edu.lv

Abstract

Wild cranberry (*Vaccinium oxycoccus* L.*)* is one of the small fruit species of commercial importance in Latvia, traditionally used in folk-medicine and food. Typically, there are wide fluctuations in yield annually and between different parts of the country. The commercial cultivation of American cranberry (*Vaccinium macrocarpon* Ait.) was successfully started during last 15 years and today Latvia is the fourth major cranberries producing country. With the increase in consumption of cranberries, widely considered being as one of the healthiest foods, it becomes important to have detailed information on the nutritional content of cranberries. The aim of this study was to compare the contents of twelve biologically essential elements (N, P, K, Ca, Mg, S,