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THE EFFECT OF INTESIFICATION FACTORS TO TOTAL ANTIOXIDANT ACTIVITY OF HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) AND LINGONBERRY (*VACCINIUM VITIS-IDAEA* L.)

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ABSTRACT

Public attention is increasingly drawn to the protective effects of natural antioxidants against civilization diseases. An important source of antioxidants are berries, which until recently has received little attention, but the latest research towards the right to it. The phenolic profile and quantitative composition of blueberries as well as the corresponding antioxidant activity of blueberries is well documented. The aim of this paper was the determination of the relationship between different methods of fertilization and total antioxidant activity of six selected varieties of blueberries and five varieties of lingonberries. Each sample of blueberry varieties (Bluejay, Nelson, Bluecrop, Patriot, Berkeley and Brigitta) and lingonberry varieties (Koralle, Ida, Sanna, Linnea and Sussi) were collected from the research station Krivá in Orava. The values of total antioxidant activity of the extracts of studied varieties of blueberry after organic fertilization ranged from 27.15 to 52.25 μg.mg^{-1} eq. Trolox. After mineral fertilization, the values of total antioxidant activity of the extracts of studied varieties of blueberry ranged from 21.27 to 51.00 μg.mg^{-1} eq. Trolox. In the control treatment, the values of total antioxidant activity of the extracts of studied varieties of blueberry ranged from 26.99 to 54.15 μg.mg^{-1} eq. Trolox. The values of values of total antioxidant activity of the extracts of studied varieties of lingonberry after organic fertilization ranged from 42.49 to 60.27 μg.mg^{-1} eq. Trolox. After mineral fertilization, the values of total antioxidant activity of the extracts of studied varieties of lingonberry were in the interval from 38.85 to 55.15 μg.mg^{-1} eq. Trolox. In the variant without fertilization, the values of total antioxidant activity of the extracts of studied varieties of lingonberry ranged from 37.16 to 65.11 μg.mg^{-1} eq. Trolox. The application of organic fertilizer has a positive effect to increasing values of antioxidant activity in blueberries and lingonberries. It should be noted that the value of the total antioxidant activity is significantly influenced by the rainfall.

Keywords: cultivars; blueberries; lingonberries; antioxidant capacity; organic fertilizer; mineral fertilizer

INTRODUCTION

Rational and efficient use of acid, low fertile soils below the mountain areas in Slovakia is currently very difficult. Due to negative changes in the environment and lifestyle of today's population, is rapidly increasing diversity of lifestyle diseases. Therefore, in many countries is preferred "Food for the Future", supporting health food, and their place in the food system and health is very important. They are preserving foods as much as possible the original biologically valuable substances, produced in an environmentally friendly way.

Public attention is increasingly drawn to the protective effects of natural antioxidants against civilization diseases. An important source of antioxidants are berries, which until recently has received little attention, but the latest research towards the right to it. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid **(Larson, 1988; Hudson, 1990; Hall and Cuppett, 1997)**. Thus, the interest in natural antioxidants has increased considerably **(Löliger, 1991)**. The high content of flavonoids, which include anthocyanins, is one indicator of antioxidant activity. Antioxidative properties of anthocyanins protect cells of the body against harmful action of oxygen radicals **(Poráčová et al., 2011)**.

From the biological point of view is an antioxidant a compound that at low concentrations in the reaction with reactive forms a relatively stable and non-toxic products, thereby protecting cells, tissues and entire organism against oxidative damage by free radicals **(Šilhár et al., 2004)**. "Antioxidant power" is the term food as a gate capacity of the human body against the action of free radicals, preventing degenerative diseases are resulting from continuous operation of the oxidative stress **(Di Majo et al., 2008)**. Result antioxidant activity is protection of biologically important molecules and, ultimately, cells,

tissues and the whole organism from oxidative damage by free radicals. The antioxidants can protect the fetus from damage and mutations prevent cancer development, protect certain enzymes vitamins **(Kyselovič, 2002)**.

Lingonberry (*Vaccinium vitis-idaea* L.) and blueberry (*Vaccinium corymbosum* L.) are flowering plants belonging to the large genus of *Vaccinium*. Lingonberry is small, thickly branched, has not deciduous perennial shrub and is closely related to the blueberry. Blueberries and lingonberries is worldwide famous as the healthy and desirable fruit and are one of the richest sources of antioxidants in our diet **(Mendelová at al., 2013; Kähkönen et al., 1999)**. Currently, the consciousness receiving fruit lingonberries and blueberries due to the high amounts of phenolic compounds, and hence a high antioxidant activity **(Prior et al., 1998)**. Small forest fruits, for example: blueberries, lingonberries, strawberries and blackberries **Beattie et al., (2005)** called "food superstars" with a protective effect against heart disease, cancer, and aging. Bioactive wild blueberry extract are full of anthocyanins and proanthocyanidins and have a significant antioxidant activity **(Smith et al., 2008)**. Blueberry and lingonberry extract prevent against the oxidation of lipids in liposomes, and to diminish the level of LDL cholesterol **(Kale et al., 2006)**.

Antioxidant capacity of blueberries is influenced by various factors (temperature, pH and oxidation) and is compared with raspberries and wild strawberries about three times higher, but the vitamin C content of about 4-fold lower **(Kalt et al., 1999)**. **Prior et al., (1998)** considered blueberries as one of the richest sources of antioxidant phytonutrients and have also confirmed the linear relationship between antioxidant capacity and total anthocyanins and polyphenols. Chemically, the antioxidants are considered to be all substances which prevent the oxidation of other compound with reactive (oxidant) that is itself oxidized. The result of antioxidant activity protect biological important molecules, and ultimately cell, tissue, and whole organism from oxidative damage species **(Ďuračková, 1998)**.

The aim of this work is to provide information on the impact of intensification factors for antioxidant activity in fruit extracts of lingonberry and blueberry.

MATERIAL AND METHODOLOGY

The experiments were based on the research station Krivá in the Orava region, which is located in the north of Slovakia. The average of temperature in this area is 6 °C and rainfall is 900 mm. The experimental area with varieties of lingonberries and blueberries is located on a slope with 10° inclination and NW exposure at an altitude of 628 – 634 meters. In the experiment we watched five varieties of lingonberry (Koralle, Ida, Sanna, Linnea and Sussi) and 6 varieties of blueberry (Bluejay, Nelson, Bluecrop, Patriot, Berkeley and Brigitta). Fruit samples for analyses were collected in August, years 2013 – 2014 and subsequently analysed at the Slovak Agricultural University in Nitra.

Lingonberries and blueberries were fertilized:

Fertilization was carried out in two different variants (mineral and organic fertilizer). The third variant (control) was without fertilizing. Fertilization by mineral fertilizers (ammonium sulfate, potassium sulfate, and superphosphate) was carried out in the spring of the five varieties of the first variant, in a ratio of 30 kg of N, 10 kg P and 30 kg of pure nutrients.$ha^{-1}.year^{-1}$. Nitrogen fertilization was divided into two parts. Half of the total dose in the spring (in the first half of April) and the other half at the end of June. P and K have been applied in one dose during each spring. Five varieties in the second variant was fertilized with Hoštický organic fertilizer with application at the beginning of vegetation (in the first half of April) at 1 kg per 10 m^2 and during the vegetation period (in the second decade of June) dose of 0.8 kg to 10 m^2. Hoštický organic fertilizer containing fermented cow and horse manure, horn and natural guano coming from the droppings of seabirds. It is a natural product containing 5% of N, 3.5% P_2O_5, K_2O 1% and 0.5% MgO.

Evaluation of the values of antioxidant activity in the fruits of lingonberry and blueberry:

Method PCL (photochemiluminesce) - The combination of fast photochemical generation of superoxide radicals with sensitive luminometric detection. It was measured luminescence of luminol remaining after the reaction of radicals with antioxidants of the samples. It allows the measuring of antioxidant activity of the hydrophilic (ACW) and lipophilic components (ACL).

ACW - aqueous extract; calibration for solutions from 0.5 to 4.0 nmol AK / 10 μL.

ACL - methanol extract; calibration for solutions from 0.5 to 4.0 nmol Trolox / 10 μL.

Repeatability RSD = 9.8% for n = 6

RESULTS AND DISCUSSION

Table No. 1 and Fig. 1 and 2 show the results of the average values of total antioxidant activity in six varieties of blueberry in various variants of fertilization.

During fertilization by Hoštický organic fertilizer (OF), the highest average value of antioxidant activity (AA) in year 2013 of blueberry had a variety Bluejay (32.6 $\mu g.mg^{-1}$ eq. Trolox) and the lowest variety Bluecrop (27.15 $\mu g.mg^{-1}$ eq . Trolox). In year 2014, during fertilization by OH, the highest average value AA had a variety Bluecrop (52.25 $\mu g.mg^{-1}$ eq. Trolox) and the lowest variety Berkeley (42.55 $\mu g.mg^{-1}$ eq. Trolox).

During fertilization by mineral fertilizer (MF), the highest AA in year 2013 of blueberry had a variety Bluejay (28.35 $\mu g.mg^{-1}$ eq. Trolox) and the lowest variety Bluecrop (21.27 $\mu g.mg^{-1}$ eq. Trolox). When fertilization variant with MF, the highest AA in year 2014 had a variety Bluejay (51 $\mu g.mg^{-1}$ eq. Trolox) and the lowest variety Nelson (36.5 $\mu g.mg^{-1}$ eq. Trolox).

The highest average value of AA was in the variant without fertilizing (WF) in year 2013 of blueberry had a variety Nelson (33.84 $\mu g.mg^{-1}$ eq. Trolox) and the lowest variety Berkeley (26.99 $\mu g.mg^{-1}$ eq. Trolox). In year 2014, the highest AA variant in WF had a variety Berkeley (54.15 $\mu g.mg^{-1}$ eq. Trolox) and the lowest variety Bluejay (38.25 $\mu g.mg^{-1}$ eq. Trolox). Our results correspond to the arguments authors **Lima et al., (2009)**, who indicated similar levels of antioxidant activity in blueberries.

Table 1 Average values of antioxidant activity (μg.mg^{-1} eq. Trolox) in selected varieties of blueberries (*Vaccinium corymbosum* L.) determined by method PCL.

Variety	Fertilization	Antioxidant activity in μg/mg eq. Trolox	
		Year 2013	Year 2014
Bluejay	WF	30.00 ±1.76	38.25 ±0.65
	MF	28.35 ±1.66	51.00 ±0.20
	OF	32.60 ±0.98	43.14 ±0.57
Nelson	WF	33.84 ±0.84	49.95 ±0.15
	MF	28.23 ±0.19	36.50 ±0.00
	OF	31.00 ±0.30	47.65 ±0.25
Bluecrop	WF	28.75 ±0,36	50.25 ±0.15
	MF	21.27 ±0.84	46.90 ±0.60
	OF	27.15 ±0.27	52.25 ±0.85
Patriot	WF	28.11 ±2.28	38.40 ±0.60
	MF	25.91 ±1.84	48.95 ±0.25
	OF	29.15 ±0.12	50.25 ±0.55
Berkeley	WF	26.99 ±0.02	54.15 ±0.55
	MF	26.83 ±2.34	48.70 ±1.10
	OF	30.92 ±0.50	42.55 ±0.05
Brigitta	WF	27.71 ±0.38	47.75 ±0.45
	MF	25.47 ±1.12	48.90 ±0.30
	OF	29.40 ±1.70	49.80 ±1.10

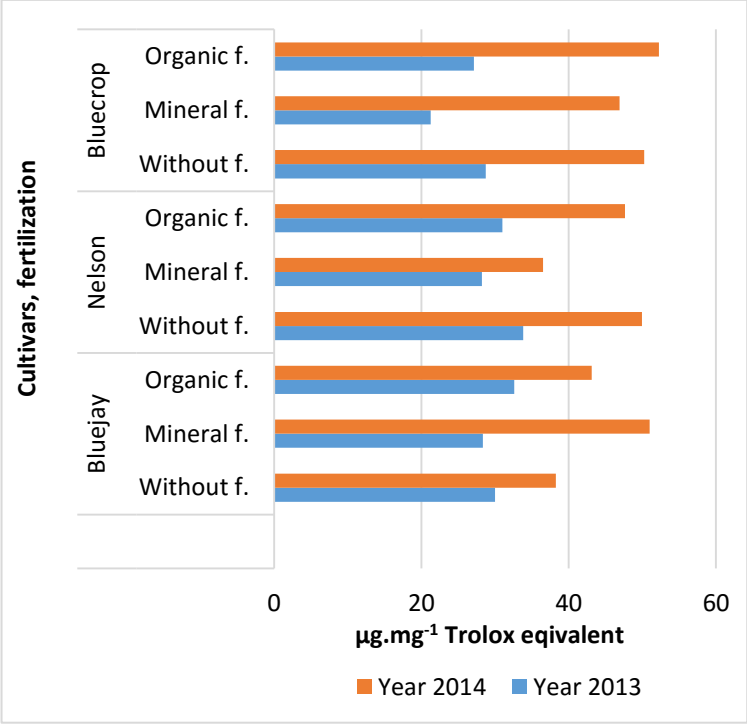

Figure 1 Average values of antioxidant activity (μg.mg^{-1} eq. Trolox) in selected varieties of blueberries (*Vaccinium corymbosum* L.).

From the average values of antioxidant activity in the variant with the application of organic fertilizer may be made the following order monitored varieties of blueberries: Bluecrop > Patriot > Brigitta > Nelson > Bluejay > Berkeley.

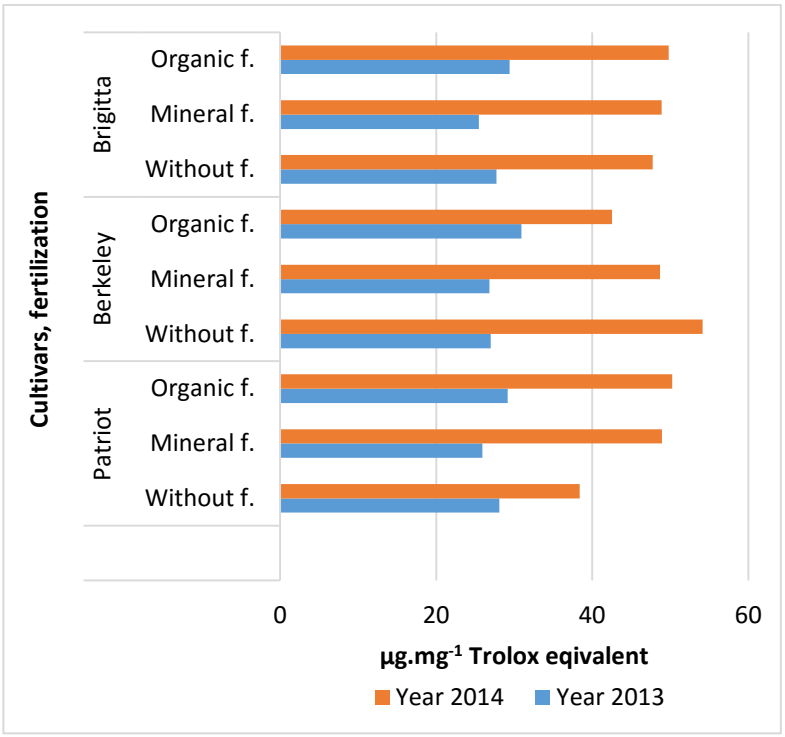

Figure 2 Average values of antioxidant activity (µg.mg^{-1} eq. Trolox) in selected varieties of blueberries (*Vaccinium corymbosum* L.).

Table 2 Average values of antioxidant activity (µg.mg^{-1} eq. Trolox) in selected varieties of lingonberries (*Vaccinium vitis-idaea* L.) determined by method PCL.

Variety	Fertilization	Antioxidant activity in µg/mg eq. Trolox	
		Year 2013	Year 2014
Koralle	WF	37.32 ±1.38	38.13 ±0.52
	MF	44.36 ±1.02	43.37 ±1.86
	OF	44.33 ±1.80	47.59 ±1.78
Ida	WF	65.11 ±1.43	56.33 ±2.51
	MF	46.42 ±0.58	55.15 ±0.14
	OF	60.27 ±1.62	48.74 ±0.66
Sanna	WF	38.61 ±1.64	51.34 ±1.28
	MF	46.57 ±0.71	54.08 ±1.51
	OF	50.12 ±1.14	53.87 ±3.11
Linnea	WF	44.77 ±1.56	51.02 ±1.76
	MF	38.85 ±0.71	52.94 ±1.31
	OF	42.49 ±1.28	54.67 ±0.32
Sussi	WF	37.16 ±1.22	42,68 ±1.14
	MF	45.37 ±0.36	52.10 ±0.08
	OF	47.26 ±0.52	49.38 ±0.64

From the average values of antioxidant activity in the variant with the application of mineral fertilizer may be made in the following order monitored varieties of blueberries: Bluejay > Berkeley > Patriot > Brigitta > Bluecrop > Nelson.

From the average values of antioxidant activity in the variant without application of fertilizer may be made in the following order monitored varieties of blueberries: Nelson > Berkeley > Bluecrop > Brigitta > Bluejay > Patriot.

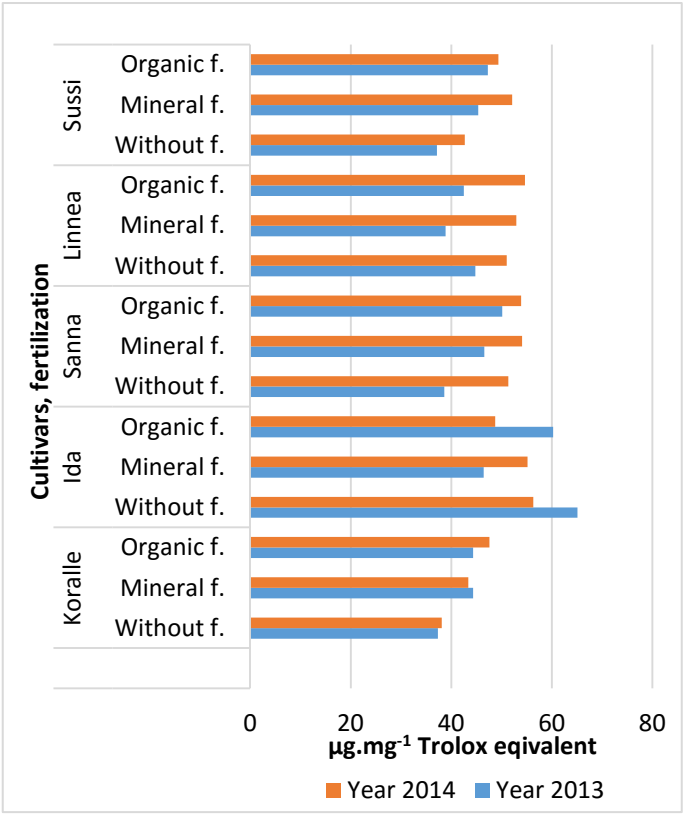

Figure 3 Average values of antioxidant activity (µg.mg^{-1} eq. Trolox) in selected varieties of lingonberries (*Vaccinium vitis-idaea* L.).

From the average values of antioxidant activity all varieties may be made in the following order monitored varieties of blueberries Berkeley > Brigitta > Nelson > Bluecrop > Bluejay > Patriot.

From the average values of antioxidant activity of blueberries all varieties may be made in the following order the fertilization methods: organic fertilization > without fertilization > mineral fertilization.

Table 2 and Figure 3 show the results of the average values of antioxidant activity in five varieties of lingonberry in various variants of fertilization.

During fertilization by Hoštický organic fertilizer (OF), the highest average value of antioxidant activity (AA) in year 2013 of lingonberry had a variety Ida (60.27 µg.mg^{-1} eq. Trolox) and lowest variety Linnea (42.49 µg.mg^{-1} eq. Trolox). In year 2014, during fertilization by OH, the highest average value AA had a variety Linnea (54.67 µg.mg^{-1} eq. Trolox) and lowest variety Koralle (47.59 µg.mg^{-1} eq. Trolox).

During fertilization by mineral fertilizer (MF), the highest AA in year 2013 of lingonberry had a variety Sanna (46.57 µg.mg^{-1} eq. Trolox) and lowest variety Linnea (38.85 µg.mg^{-1} eq. Trolox). In year 2014, during fertilization by MF, the highest AA had a variety Ida (55.15 µg.mg^{-1} eq. Trolox) and lowest variety Koralle (43.37 µg.mg^{-1} eq. Trolox).

The highest average value of AA was in the variant without fertilizing (WF) in year 2013 of lingonberry had a variety Ida (65.11 µg.mg^{-1} eq. Trolox) and the lowest variety Sussi (37.16 µg.mg^{-1} eq. Trolox). In year 2014, the highest AA variant in WF had a variety Ida (56.33 µg.mg^{-1} eq. Trolox) and lowest Koralle variety (38.13 µg.mg^{-1} eq. Trolox).

From the average values of antioxidant activity in the variant with the application of organic fertilizer may be made the following order monitored varieties of lingonberries: Ida > Sanna > Linnea > Sussi > Koralle.

From the average values of antioxidant activity in the variant with the application of mineral fertilizer may be made in the following order monitored varieties of lingonberries: Ida > Sanna > Sussi > Linnea > Koralle.

From the average values of antioxidant activity in the variant without application of fertilizer may be made in the following order monitored varieties of lingonberries: Ida > Linnea > Sanna > Sussi > Koralle.

From the average values of antioxidant activity may be made in the following order monitored varieties of lingonberries Ida > Sanna > Linnea > Sussi > Koralle.

From the average values of antioxidant activity of lingonberries all varieties may be made in the following order the fertilization methods: organic fertilization with variant> variant with mineral fertilizers > variant without fertilization.

The average value of the total antioxidant activity in cultivated varieties of blueberries was 21.5% lower than in cultivated varieties of cranberries. We confirmed with author **Zheng and Wang (2003)**, who reported that blueberries have higher antioxidant capacity than lingonberries.

CONCLUSION

The presented research aimed at the effects of two fertilizer application treatments on the total antioxidant

activity in the fruit of some varieties of highbush blueberry (*Vaccinium corymbosum* L.) and lingonberry (*Vaccinium vitis-idaea* L.). The research results showed that the antioxidant activity was higher at the treatments with an organic fertilizer application. It was concluded that the values of total antioxidant activity can be influenced by the fruit variety, the soil properties (e.g. grain size, nutrient content, humus) and the climatic conditions.

1. The most positive effects on the overall mean antioxidant activity in the high-bush blueberry fruit were found at the treatment with the "Hoštické" organic fertilizer applied. The highest values of antioxidant activity were recorded at this treatment throughout the research years (2013 and 2014). The least positive impact on the overall mean antioxidant activity of high-bush blueberry fruit was recorded at the treatment with mineral fertilizer application.
2. From the point of view of average values of antioxidant activity of the extracts in year 2013 and year 2014 may be made in the following order monitored varieties of blueberries: Berkeley > Brigitta > Nelson > Bluecrop > Bluejay > Patriot.
3. The most positive impact on the total mean antioxidant activity in the fruit of lingonberry was found also at the treatment with the "Hoštické" organic fertilizer application, what may be considered a notable factor of influence on the human body. The least positive effects on the total antioxidant activity were recorded at the zero fertilizer application treatment (the control).
4. From the point of view of average values of antioxidant activity of the extracts in year 2013 and year 2014 may be made in the following order monitored varieties of lingonberries: Ida > Sanna > Linnea > Sussi > Koralle.
5. The high value antioxidant activity was influenced by rainfall, which was reflected in year 2014, when it had been raining a lot during the growing season compared to 2013. This rainfall was reflected up to twice as high values in most varieties of blueberries and lingonberries.

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