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ORIGINAL PAPER

EFFECT OF MYCORRHIZATION AND VARIETY ON THE CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF SEA BUCKTHORN BERRIES*

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Abstract

As well as being a rich source of nutrients, sea buckthorn berries contain large amounts of biologically active substances. Numerous studies have confirmed that the plants colonized by arbuscular mycorrhizal fungi have greater resistance to a shortage of nutrients, soil salinity, high temperature and prolonged drought. Currently, there is little information on the influence of mycorrhization on the chemical composition and antioxidant properties of these plants. This study was conducted in 2014 and 2015 at the Experimental Station in Lipnik, Poland. The levels of essential nutrients, dietary fiber fractions, minerals, antioxidants, and the ability to neutralize ABTS radical cations in sea buckthorn berries were investigated. The berries of mycorrhized sea buckthorns had more crude fiber (15%) and total carbohydrates (2%), K (19%), Ca (28%), Na (16%), Zn (2%), and Cu (31%), they contained lower levels of antioxidants (polyphenols 6%, carotenoids 25%, flavonoids 14%), and had a lower ability to neutralize the ABTS radical cation (12%) compared to the untreated control. The variety Hergo had more favourable chemical composition, containing more protein (16%), crude fiber (18%), ash (1%) and total carbohydrates (14%) as well as a better mineral composition than the variety Habego. This study did not confirm unambiguously the beneficial effect of mycorrhization on the quality of sea buckthorn berries. Mycorrhization increased the levels of various nutrients in the berries, yet at the same time it decreased the antioxidant potential.

Keywords: ABTS, fibre fractions, *Hippophae rhamnoides* L., mineral components, vitamins.

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INTRODUCTION

Although the taste and nutritional characteristics of sea buckthorn berries are well documented (ZEB, MALOOK 2009, GUPTA et al. 2011), there is little information on changes in nutritional properties and bioactive substances in the berries induced by agrotechnical activities intended to increase the quality of the yield. Previous reports relate primarily to the impact of the origin of sea buckthorn, climate and extraction methods (ZEB, MALOOK 2009). The need to respect the EU environmental directives by its Member States, more environmentally friendly principles of crop production, as well as the changing expectations of consumers, have forced growers to use novel techniques in crop protection to replace the use of chemicals. One of the more effective solutions is mycorrhization, not only beneficial to the various parameters of plant growth, but also to crop quality. Colonization of the roots by various arbuscular mycorrhizal fungi (AMF) increases the concentration of oil in plants, e.g. in oregano (*Origanum vulgare* L.) and basil (*Ocimum basilicum* L.) (COPETTE et al. 2006, KHAOSAAD et al. 2006). Mycorrhized plants, e.g. alfalfa (*Medicago sativa* L.) and soybeans (*Glycine max* L.), show increased levels of flavonoids (MORANDI 1996, LAROSE et al. 2002). Lower concentrations of cadmium have been found in mycorrhized common grape vine (*Vitis vinifera* L.) by KARAGIANNIDIS, NIKOLAOU (2000). On the other hand, KUMAR et al. (2015) show that inoculation with arbuscular mycorrhizal fungi (AMF) does not reduce the detrimental effect of Cd on growth and productivity.

Our research hypothesis assumed that the use of AMF and the plant variety studied determine the chemical composition and antioxidant activity of sea buckthorn berries. We conducted a field experiment to test the impact of symbiotic mycorrhizal fungi on the chemical composition and antioxidant activity of the berries of two sea buckthorn varieties.

MATERIALS AND METHODS

The study was conducted in 2014 and 2015 at The Experimental Station in Lipnik, Poland. According to the Polish Soil Classification (2011), the soil in which the experiment was carried out belonged to typical rusty soils, classified as Haplic Cambisol (IUSS WRB 2015). The analysis of soil minerals showed moderate levels of Mg and K, and high P levels. The concentration of metals in the soil did not exceed limit values (Ordinance of the Ministry of the Environment 2002). The experiment was designed according to a completely randomized method in five replications (one shrub – one repeat). The subject of the study included two and three year old shrubs; female varieties Habego and Hergo. Shrubs were planted in 4×3 m spacing. The size of a single plot was 12 m^2. Mycorrhization was conducted with ectomycorrhizal mycelium, which is symbiotic for plants of the olive family. The isolate containing symbiotic mycorrhizal fungi (*Glomus* spp., *Gigaspora* spp., *Pochonia* spp., *Lecanicillum* spp.) and the root bacteria (*Bacillus* spp.) was obtained from the natural ecosystems in Croatia. A dose of 15 mL was applied in two places in the root zone of the plants in the first year of the experiment. The mycelium contained some hydrogel (ensuring moisture essential for the initial fungi development). The experiment was set up according to the factors: (1) variety – Habego and Hergo; (2) application of mycorrhization in some of the plants: half of the plants of each variety (5 specimens) were subject to mycorrhization.

The colonization of mycorrhiza was observed under an optical microscope. There was no colonization of mycorrhizal fungi on the roots of the control plants.

The berries were harvested manually in the third week of August and kept in a refrigerator at 4°C (24 h) until pre-treatment and drying. The initial moisture content was close to 793 g kg^{-1} (fresh weight, Table 1).

Dry weight, crude protein (N · 6.25), crude fat, crude fibre, crude ash and total carbohydrates were determined by AOAC (2012). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined using the detergent method according to Van Soest et al. (1991) on an ANKOM 220 Fibre Analyzer (USA). Hemicellulose (HCEL) was calculated as the difference between NDF and ADF, and cellulose (CEL) – as the difference between ADF and ADL.

The concentration of P was determined by the colorimetric method in a Specol 221 apparatus. An Atomic Absorption Spectrometer (ASA) (iCE 3000 Series, Thermo Fisher Scientific) was used to determine K, Na and Ca by

Table 1

Chemical composition of sea buckthorn berries (g kg^{-1} d.w.)

Item	Mycorrhizal effect		*P*-value	Genotype effect		*P*-value
	control	mycorrhiza		Habego	Hergo	
Moisture (g kg^{-1}f. w.)	$794.8^a \pm 12.1$	$791.7^b \pm 20.6$	0.001	$807.4^a \pm 2.6$	$779.1^b \pm 6.1$	0.000
Dry weight (g kg^{-1} dried berries)	$954.6^a \pm 1.5$	$952.9^a \pm 1.1$	0.130	$954.5^a \pm 1.4$	$953.1^a \pm 1.3$	0.170
Crude protein	$92.3^a \pm 3.3$	$93.3^a \pm 1.4$	0.102	$85.9^b \pm 4.1$	$99.6^a \pm 5.1$	0.000
Crude ash	$40.6^a \pm 1.1$	$40.8^a \pm 0.2$	0.312	$40.6^b \pm 1.1$	$41.1^a \pm 0.5$	0.015
Crude fat	$413.8^a \pm 47.2$	$394.7^b \pm 42.4$	0.000	$443.1^a \pm 13.5$	$365.5^b \pm 8.7$	0.000
Crude fibre	$63.1^b \pm 7.3$	$72.7^a \pm 6.1$	0.000	$62.2^b \pm 6.3$	$73.6^a \pm 4.9$	0.000
Total carbohydrates	$390.1^b \pm 35.7$	$398.4^a \pm 24.0$	0.020	$368.5^b \pm 11.1$	$420.1^a \pm 2.0$	0.000

d.w. – dry weight, f.w. – fresh weight; Mean values with the same letter in each line are not significantly different.

emulsion flame spectroscopy, and Mg, Zn, Fe, Pb, Cr, and Cu by absorption flame spectroscopy.

For total polyphenols, total flavonoids, and antioxidant activity determination, methanol extracts were prepared according to Kumaran, Karunakaran (2007). The total phenolic content of the plant extracts was determined using the Folin-Ciocalteu reagent (YU et al. 2002). The total flavonoid content was determined according to Kumaran, Karunakaran (2007) using quercetin as a reference compound. The antioxidant capacity was determined with the Trolox Equivalent Antioxidant Capacity (TEAC) method described by Re et al. (1999). The radical used was 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid, ABTS) with the absorption maximum of 734 nm. Carotenoids were determined according to Lichtenthaler, Welburn (1983). Tocopherols were determined by the Prieto et al. (1999) method. A Shimadzu UV-1800 spectrophotometer was used. The content of thiamine (B1), riboflavin (B2), ascorbic acid (C), and niacin (PP) in the extracts was measured using the high-performance liquid chromatographic (HPLC) method described by Kłódka et al. (2008).

All calculations were made using an ANOVA. The significance of differences between means was compared by the Tukey multiple range tests. The admissible error for determinations of chemical components was 5%. Results are presented as the mean of the two years of the experiment ± SD (standard deviation). All samples were analyzed in triplicate. Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

The dry weight of the berry was on average 20% of the fresh weight (Table 1). The examined determinants did not differentiate dry weight. The tested berries contained on average of 92.8 g protein kg^{-1} d.w. It was almost twice as much as the protein level found by Selvamuthukumar, Farhath (2014) in sea buckthorn berries originating in India (47 g kg^{-1} d.w.). The differences probably result from the genotype and the location (environmental conditions). Zheng et al. (2012) report that sea buckthorn shows varied chemical composition under the influence of genetic and environmental factors (soil conditions and weather conditions). In the present study, the use of mycorrhizae had no effect on the protein content in sea buckthorn berries.

The berries of the studied varieties of sea buckthorn were characterized by a relatively high content of protein compared to other berry varieties. For example, Rodrigues et al. (2009) show 18.5 g protein kg^{-1} d.w. in golden berries (*Physalis peruviana* L.), and only 9 g kg^{-1} d.w. in strawberry (*Fragaria vesca* L.) and acerola (*Malpighia glabra* L.). A study by Ahmad et al. (2015) shows 32.8 g protein kg^{-1} d.w. and 4.37 g kg^{-1} d.w. in raspberries (*Rubus niveus*, *Rubus ellipticus*) and 65.6 g kg^{-1} d.w. in blackberries (*Rubus ulmifolius*).

Sea buckthorn is one of the few plants that accumulate lipids in berries (Yang, Kallio 2001). In the studied varieties of sea buckthorn, fat averaged 404.3 g kg^{-1} d.w., which is much more than in raspberries (*R. niveus* – 11.0 g kg^{-1} d.w., *R. ellipticus* – 27.3 g kg^{-1} d.w.) and blackberries (*R. ulmifolius* – 47.3 g kg^{-1} d.w.) (Ahmad et al. 2015). Importantly, the fat content in the berries of mycorrhized sea buckthorn was significantly lower than in the control (by 4.6%).

Sea buckthorn berries are also a source of minerals, measured as crude ash. The greater the amount of crude ash, the higher the mineral content in the tested material. The tested berries contained on average 40.7 g kg^{-1} d.w. of crude ash, which confirms the results obtained by Selvamuthukumaran, Farhath (2014). The berries of var. Hergo had on average 1.2% more crude ash than var. Habego. Mycorrhization did not affect the content of crude ash.

Carbohydrates are a major component of dry matter in plant food products. In this study, total carbohydrates (TC) were the main component of dry weight (in addition to lipids), with an average of 394.3 g kg^{-1} d.w. In the study by Selvamuthukumaran, Farhath (2014) this figure is 1.5 times higher. The Hergo variety had a greater levels of TC than var. Habego. Significantly, mycorrhized berries had a higher TC than control.

Mycorrhization of the selected sea buckthorn varieties increased the crude fiber content in the berries by 13.2%. The increase was more pronounced in the Hergo variety. Selvamuthukumar, Farhath (2014) found an even higher level of crude fiber, at 100.32 g kg^{-1} d.w. (about 32% more than in this study), probably due to differences in weather conditions and maturity of the berries as the fiber content in the cell walls of berries changes during ripening.

High fiber content is an important reason why berries are recommended as healthy food. All the analyzed samples showed high levels of crude fiber (Table 1) and dietary fiber fractions (Figure 1), the latter being more abundant in the Hergo variety. Mycorrhization contributed to an increase in neutral detergent fraction (NDF), acid detergent fraction (ADF), hemicellulose (HCEL), and cellulose (CEL). The determined level of NDF was similar to levels found in other fruits. According to Schmidt et al. (2000), the smallest

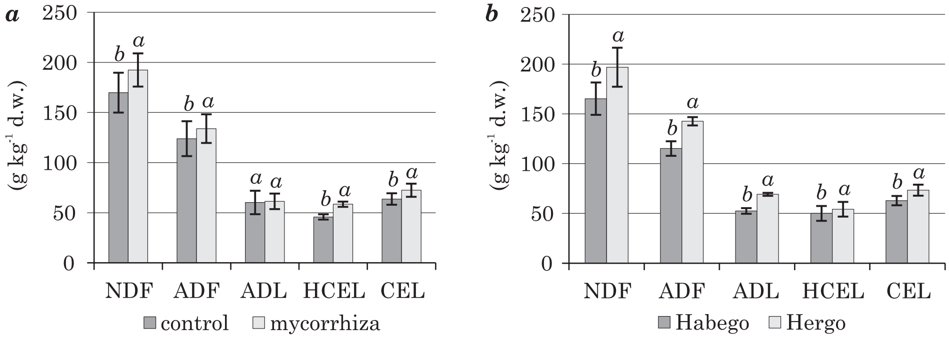

Fig. 1. Influence of mycorrhization (*a*) and variety (*b*) on fiber fraction

amount of this fraction is found in green grapes (3.27 g kg^{-1} d.w.) and the highest is in melons (220.4 g kg^{-1} d.w.). The studied sea buckthorn berries contained amounts of NDF similar to blueberry (*Vaccinium myrtillus* L.), kiwifruit (*Actinidia callosa* L.) and watermelons (*Citrullus lanatus* var. Lanatus). The average content of NDF in the studied berries was in the range typical of vegetables considered to be a very good source of dietary fiber fraction (97-219 g kg^{-1} d.w.) (Schmidt et al. 2000). The determined ADF was on average 126.9 g kg^{-1} d.w. As in the case of NDF fractions, ADF in sea buckthorn berries was similar to blueberry, kiwifruit and watermelon (Schmidt et al. 2000).

Cereal grains, herbs and vegetables are a significant source of hemicellulose (Yangilar 2013). In comparison with the above sources, sea buckthorn berries contained little of this dietary fiber fraction (Figure 1). The level of hemicelluloses averaged 52.1 g kg^{-1} d.w., with Hergo berries having 7.8% more HCEL than Habego ones. The highest level of cellulose was determined in Hergo berries, 14.4% more than in berries of the Habego variety. However, there is no information in the literature on the fraction of cellulose in sea buckthorn berries. The level of ADL, which to some extent affects the hardness of the berry, was not influenced by the process of mycorrhizal inoculation (Figure 1). It was, however, influenced by the variety, as var. Hergo berries had 24% more ADL than Habego ones. The average NDF and ADL levels were similar to those found in pumpkin fruit (Cerniauskiene et al. 2014).

Sea buckthorn berries are a rich source of vitamins, organic acids, antioxidants and minerals. The levels of macro- and microelements in the investigated sea buckthorn berries are shown in Table 2. Compared with sea

Table 2

Macro- and microelements in sea buckthorn berries

Item	Mycorrhizal effect		P-value	Genotype effect		P-value
	control	mycorrhiza		Habego	Hergo	
Macroelements (g kg^{-1}d.w.)						
P	$9.16^a \pm 0.4$	$7.11^b \pm 0.7$	0.000	$7.63^b \pm 1.3$	$8.64^a \pm 1.0$	0.000
K	$18.2^b \pm 0.2$	$21.6^a \pm 4.1$	0.000	$18.3^b \pm 0.2$	$21.6^a \pm 4.4$	0.000
Mg	$1.03^a \pm 0.1$	$1.03^a \pm 0.0$	0.814	$0.99^b \pm 0.0$	$1.1^a \pm 0.0$	0.000
Ca	$0.28^b \pm 0.0$	$0.36^a \pm 0.0$	0.000	$0.32^a \pm 0.0$	$0.32^a \pm 0.0$	0.740
Na	$0.09^b \pm 4.3$	$0.11^a \pm 28.9$	0.000	$0.10^a \pm 24.9$	$0.08^b \pm 8.3$	0.000
Microelements (mg kg^{-1}d.w.)						
Fe	$21.4^a \pm 0.6$	$20.9^a \pm 2.4$	0.282	$22.3^a \pm 0.8$	$19.9^b \pm 1.4$	0.004
Zn	$26.3^b \pm 3.2$	$26.7^a \pm 1.1$	0.000	$25.6^b \pm 2.3$	$27.5^a \pm 1.9$	0.000
Cr	$0.39^a \pm 0.2$	$0.64^a \pm 0.0$	0.093	$0.48^a \pm 0.2$	$0.54^a \pm 0.2$	0.063
Cu	$5.99^b \pm 0.9$	$7.84^a \pm 0.7$	0.024	$7.08^a \pm 1.8$	$6.74^a \pm 0.5$	0.551
Pb	nd	nd		nd	nd	

nd – not detected; Explanations under Table 1 buckthorn grown in Pakistan (Sabir, Maqsood 2005) and with other berry plants (Tosun et al. 2008), the mineral content in the tested berries was relatively high.

Mycorrhization significantly affected the concentrations of P, K, Ca, and Na. Compared to the control, the berries of mycorrhized plants contained 19% more K, 28% more Ca, and 16% more Na. The P content was 22% lower than in the untreated berries. The berries of mycorrhized sea buckthorn also had 2% more Zn and 31% more Cu. Greater levels of K and Cu than in the mycorrhized sea buckthorn berries were shown by Castellanos-Morales et al. (2010) in mycorrhized strawberries.

In comparison to the Habego variety, the Hergo variety berries had significantly more P (13%), K (18%), and Mg (11%). In turn, Hergo sea buckthorn berries contained significantly less Na (20%). Significant differences in the content of trace elements between the two varieties were found for Fe and Zn. Namely, berries of the Hergo variety contained less Fe (11%) and more Zn (7%).

The variations in mineral content in the tested berries, despite the same habitat conditions, indicate the different abilities of individual sea buckthorn varieties to absorb and accumulate minerals in their biomass, which confirms the results obtained by Katiyar et al. (1990) and Sabir, Maqsood (2005). Importantly, we found no traces of Pb in the tested samples.

In recent years, much attention has been paid to the high antioxidant properties of berries, effective in the prevention of cardiovascular diseases and some types of cancer (Guo et al. 2003). Sea buckthorn berries are a particularly rich source of antioxidants and other biologically active substances. The content of antioxidants in the investigated sea buckthorn berries is shown in Table 3.

Mycorrhization of sea buckthorn reduced the level of total flavonoids in the berries by 14%. However, at 1074.3 mg kg^{-1} d.w., the average was much

Table 3

Content of antioxidants in sea buckthorn berries

Item	Mycorrhizal effect		*P*-value	Genotype effect		*P*-value
	control	mycorrhiza		Habego	Hergo	
Polyphenols (mg GAE/kg^{-1} d.w.)	4047.9^{a} ± 240.5	3799.1^{a} ± 298.9	0.059	4006.0^{a} ± 175.4	3840^{a} ± 370.3	0.180
Total flavonoids (mg QE/kg^{-1} d.w.)	1152.5^{a} ± 94.5	996.2^{b} ± 28.2	0.000	1048.7^{a} ± 56.0	1100.0^{a} ± 140.2	0.112
Total carotenoids (mg kg^{-1} d.w.)	1907.7^{a} ± 54.8	1438.1^{b} ± 77.0	0.000	1662.9^{a} ± 219.9	1682.9^{a} ± 304.4	0.578

GAE – gallic acid equivalent, QE – quercetin equivalent; Explanations under Table 1 higher than in berries investigated by Chu et al. (2003), which contained 380 mg kg^{-1} d.w. of flavonoids. Mycorrhization also reduced the amount of carotenoids (by 25% compared to control), still very high at 1672.9 mg kg^{-1} d.w, e.g. compared to the range 530-970 mg kg^{-1} d.w., given by Pop et al. (2014) for sea buckthorn berries.

Although mycorrhization did not influence all the examined antioxidants in sea buckthorn berries, there was a visible downward trend. Likewise, despite the lack of a statistically significant difference between the varieties, slightly better antioxidant properties were found for the Hergo variety (with the exception of polyphenols, 4% less than in var. Habego). The Hergo berries contained 5% more total flavonoids and 1% more carotenoids than the Habego ones.

A significantly higher antioxidant activity measured by ABTS assay was found in the untreated sea buckthorn berries (Figure 2). The determined

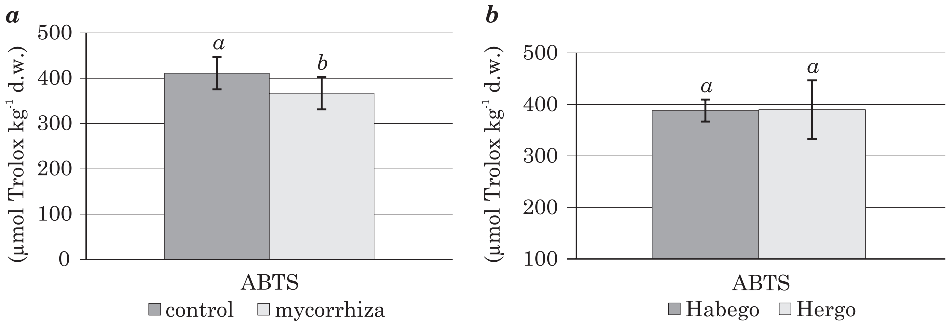

Fig. 2. Influence of mycorrhization (*a*) and variety (*b*) on antioxidant activity

antioxidant activity of the sea buckthorn berries was still higher than in wild edible fruits of the Indian Himalayan Region reported by Bhatt et al. (2017) (*Fragaria indica*, *Prunus armeniaca*, *Pyracantha crenulata*, *Terminalia chebula*, *Phyllanthus emblica*).

Although we found no statistically significant dependence between antioxidant activity and the levels of polyphenols and total flavonoids, we did observe a growing trend with regard to antioxidant activity along with an increase in the content of antioxidants in the untreated sea buckthorn berries (Table 3, Figure 2*a*), which confirms the results of Sytar et al. (2016).

The tested sea buckthorn berries had high levels of both fat- and water-soluble vitamins (Table 4). These levels did not depend on mycorrhization, although we did find differences between the varieties in the levels of tocopherols and L-ascorbic acid (Table 4). In a study by Andersson et al. (2008), the total tocopherol content in sea buckthorn berries ranged from 260.4 to 1173.8 mg kg^{-1} d.w., with a mean value of 485.1 mg kg^{-1} d.w. In the present study mycorrhization contributed to a 13.2% reduction in the content of tocopherols in sea buckthorn berries relative to the control. These levels also

Table 4

Content of vitamins in sea buckthorn berries (mg kg-1d.w.)

Item	Mycorrhizal effect		P-value	Genotype effect		P-value
	control	mycorrhiza		Habego	Hergo	
Tocopherols	1098.2^{a} ± 225.9	953.1^{b} ± 65.0	0.000	954.1^{b} ± 64.6	1097.2^{a} ± 226.8	0.000
L-ascorbic acid	3959.3^{a} ± 649.7	3344.2^{b} ± 46.6	0.000	3352.6^{b} ± 29.4	3951.0^{a} ± 660.1	0.000
Thiamine	24.21^{a} ± 1.4	24.7^{a} ± 2.1	0.645	24.66^{a} ± 2.20	24.26^{a} ± 1.6	0.717
Riboflavin	66.21^{a} ± 1.1	65.77^{a} ± 1.7	0.640	65.9^{a} ± 1.2	66.0^{a} ± 1.6	0.921
Niacin	149.5^{a} ± 11.5	138.3^{b} ± 9.2	0.039	139.0^{a} ± 5.9	148.8^{a} ± 14.1	0.065

explanations under Table 1

depended on the variety. The Hergo variety contained 13% more of this nutrient than var. Habego did.

Sea buckthorn berries are well known for their extraordinarily high levels of L-ascorbic acid, from 2630 mg kg^{-1} to 3990 mg kg^{-1} d.w (Arif et al. 2010). In our study as well, L-ascorbic acid reached relatively high levels in the sea buckthorn berries, on average 3651.8 mg kg^{-1} d.w. L-ascorbic acid, 15% more in var. Hergo. The ascorbic acid content in the berries of sea buckthorn was thus higher than in most fruits of berry plants (2137.8 g kg^{-1} d.w. in blackberry, *R. discolor*; 2063.7 g kg^{-1} d.w. in raspberry, *R. idaeus*) (Dujmović, Purgar et al. 2012). Vitamin C in sea buckthorn is very durable and its levels do not decrease as a result of a thermal treatment; sea buckthorn has no enzymes such as ascorbate oxidase that degrade this vitamin (Gutzeit et al. 2008).

None of the studied factors affected the levels of vitamins B1, B2 and B3 in the tested sea buckthorn berries (24.46, 65.99, and 143.9 mg kg^{-1} d.w. respectively), which were around ten-fold higher than in blackberries (*Rubus* spp.) examined by Ağaoğlu et al. (2006).

CONCLUSIONS

The results confirm that sea buckthorn berries are a rich source of nutrients and bioactive antioxidant compounds, whose levels vary depending on the location, climate, and agronomic methods used. Mycorrhization of sea buckthorn increased the levels of crude fiber and total carbohydrates, K, Ca, Na, Zn and Cu in its berries. However, at the same time it reduced the levels of antioxidants and the ability to neutralize ABTS radical cations. Mycorrhization increased the neutral detergent fraction, acid detergent fraction, hemicellulose and cellulose. The Hergo variety had higher protein, crude fiber, and crude ash levels, and more favourable mineral composition.

Although there was no statistically significant difference in the antioxidant capacity and the ability to neutralize the ABTS radical cation between the two varieties, higher values of the analyzed traits were found in var. Hergo. This study did not unambiguously confirm the beneficial effects of mycorrhization on the chemical composition and antioxidant properties of sea buckthorn berries. Although it increased the levels of some nutrients in the berries, at the same time it also reduced their antioxidant potential.

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