Journal of Central European Agriculture, 2013, 14(3), p.1210-1219 DOI: 10.5513/JCEA01/14.3.1328

Analysis of Biologically Active Substances in Bilberry (*Vaccinium myrtillus* L.) in Selected Natural Localities of Slovak Republic

Analýza biologicky aktívnych látok v brusnici ļ uļ oriedkovej (*Vaccinium myrtillus* L.) z rozliļ ných prírodných lokalít Slovenskej republiky

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Abstract

The bioactive substances such as total polyphenols, anthocyanins, quercetin, chlorogen acid, pterostilbene and antioxidant activity in samples of bilberry (*Vaccinium myrtillus* L.) from different natural localities of the Slovak Republic ï Oġļ adnica and Liesek (northwestern Slovakia), Poprad, Keģmarok, Stará ōubovŕa (northeastern Slovakia) and one sample of blueberry (*Vaccinium corymbosum* L.) from local supermarket was determined. The total of polyphenols ranged from 33772.5 ± 737.99 mg*kg^{-1} dw (Keģmarok) to 9685.0 ± 958.63 mg*kg^{-1} dw (supermarket). The content of total antocyanins was detected in a range from 5578.0 mg*kg^{-1} dw in a sample from the supermarket to 2887.75 mg*kg^{-1}dw from Keģmarok locality. The highest quercetin content was detected in the sample from Liesek locality (6.79 ± 0.36 mg*kg^{-1} dw), then in Stará ōubovŕa (6.03 ± 0.31 mg*kg^{-1}dw) and Keģmarok (5.83 ± 0.39 mg*kg^{-1}dw), respectively. The content of chlorogenic acid ranged from 53.19 ± 1.33 mg*kg^{-1}dw to 5.20 ± 0.16 mg*kg^{-1}dw. Higher amounts were detected in samples harvested from Poprad locality and the order follows: Poprad > Keģmarok > Stará ōubovŕa > Oġļ adnica > supermarket > Liesek. The content of pterostilben varied between 2.162 ± 0.217 mg*g^{-1} dw in the sample from the supermarket and 1.274 ± 0.312 mg*g^{-1} dw in the sample from Stará ōubovŕa. The highest values of antiradical activity were found in the sample from Keģmarok (81.180 ± 2.020 % inhibition). Based on these measures a conclusion is that geographical location of bilberry harvest was functional in relation to levels of bioactive substances, plus other factors such as weather, ripeness and harvest technique may have a contributory role. From these results, it can be determined, that bilberry contains a significant

volume of bioactive substances ï highly effective during preventive and supportive therapy of chronic diseases such as cardiovascular diseases, hypercholesterolemia, diabetes mellitus and others.

Keywords: antioxidant activity, anthocyanins, bilberry (*Vaccinium myrtillus* L.), chlorogen acid, locality, pterostilbene, quercetin, Slovak Republic, total polyphenols

Abstrakt

Práca prináša výsledky hodnotenia antioxidaļnej aktivity a obsahu bioaktívnych látok ako sú celkové polyfenoly, antokyaníny, kyselina chlorogénová, quercetin a pterostilben vo vzorkách ļuļoriedky obyļajnej (*Vaccinium myrtillus* L.). Hodnotené vzorky boli zhromažďované z vybraných prírodných lokalít severného Slovenska (Oľļadnica, Liesek, Poprad, Kežmarok a Stará ĺubovňa), jedna vzorka bola z ponuky miestnych supermarketov, krajina pôvodu Uruguaj. Na základe získaných meraní a výsledkov možno konštatovaŤ že na obsah bioaktívnych látok mala vplyv lokalita pôvodu (rastu) ļuļoriedky, priļom sa prejavil aj vplyv ďalších faktorov ako sú poveternostné podmienky, stupeň zrelosti a spôsob zberu plodov. Získané výsledky možno interpretovaŤ ako potvrdenie toho, že ļuļoriedky obsahujú významné množstvo bioaktívnych látok, ktorých vplyv možno hodnotiŤ ako vysoko efektívny v prevencii a tiež aj v podpornej liečbe chronických ochorení neinfekčného pôvodu, ako sú kardiovaskulárne ochorenia, hypercholesterolémia, diabetes mellitus a ďalšie.

Kľúčové slová: antokyaníny, antioxidačná aktivita, celkové polyfenoly, ļuļoriedky (*Vaccinium myrtillus*, L.), kyselina chlorogénová, lokality, pterostilben, quercetin, Slovenská republika

Detailný abstrakt

V práci sme sledovali vybrané kvalitatívne parametre plodov brusnice ļuļoriedkovej (*Vaccinium myrtillus* L.) z rozličných lokalít Slovenskej republiky. Intenzifikáciou a poľnohospodárskou produkciou v horských a podhorských oblastiach v rokoch 1960 ï 1989 došlo k poklesu prírodných lokalít ļuļoriedok. Navyše sa viaceré prírodné lokality nachádzajú v oblastiach s rôznym stupňom ochrany, kde je zber obmedzený alebo zakázaný. Okrem lesných ļuļoriekok *(Vaccinium myrtillus* L.) sú v súčasnosti na trhu dostupné aj rozličné kultivary záhradných ļuļoriekok (*Vaccinium corymbosum* L.) u nás známych aj ako kanadské ļuļoriedky. Keďže domáca produkcia je nedostatočná, na trhu sú dostupné tieto plody dovezené od pestovateľov z Kanady a z krajín Ameriky, ktorí sú zároveň najväčšími producentami. Vzhľadom k tomu, že pre širokú verejnosŤ sú na trhu predovšetkým takéto zdroje, zaradili sme do výskumu aj takto získanú vzorku (vzorka č. 6 ï *V. corymbosum*, krajina pôvodu Uruguaj) a porovnávali sme obsah bioaktívnych látok so vzorkami získanými z prírodných lokalít Slovenska. Zistili sme, že vplyv lokality výskytu ļuļoriedky na obsah celkových polyfenolov, antokyanínov, quercetínu, pterostilbenu, kyseliny chlorogénovej ako aj na celkovú antioxidačnú aktivitu je štatisticky vysoko preukazný, priļom možno predpokladaŤ uplatnenie aj ďalších faktorov, ako sú priebeh počasia, stupeň zrelosti, spôsob zberu a i. Najvyššší obsah celkových polyfenolov bol zistený vo vzorke z lokality Kežmarok (33772,5 mg*kg^{-1} sušiny). Medzi

touto hodnotou a vġetkými ostatnými boli zistené ġtatisticky významné rozdiely ($p<0,05$). Rozdiely v obsahu medzi lokalitami Stará ōubovŕa (27737,5 mg*kg^{-1} suġ.) a Poprad (27627,5 mg*kg^{-1} suġ.) neboli signifikantné ($p>0,05$). Najniġġí obsah celkových polyfenolov bol vo vzorke z obchodnej siete (9685,5 mg*kg^{-1} suġ.). Obsah antokyanínov bol zistený v rozpätí od 5578,0 mg*kg^{-1} suġ. vo vzorke z obchodnej siete do 2887,75 mg*kg^{-1} suġ. z lokality Keġmarok a medzi týmito hodnotami bol ġtatisticky významný rozdiel ($p < 0,05$). Druhá najvyġġia hodnota obsahu antokyanínov bola vo vzorke z lokality Poprad (3815.25 mg*kg^{-1} suġ.), za ŕou nasledovali lokality Stará ōubovŕa (3353,0 mg*kg^{-1} suġ.), Liesek (3135,75 mg*kg^{-1} suġ.) a Oġļ adnica (3039,75 mg*kg^{-1} suġ.). Medzi obsahom quercetínu z lokality Liesek (6,79 mg*kg^{-1} suġ.), ktorý bol zároveň najvyġġí a v poradí s ňalġími lokalitami Stará ōubovŕa (6,185 mg*kg^{-1} suġ.) a Keġmarok (5,980 mg*kg^{-1} suġ.) neboli ġtatisticky významné rozdiely. Signifikantné rozdiely boli zistené medzi vzorkou z lokality Poprad (5,210 mg*kg^{-1} suġ.) a vzorkou z obchodnej siete (2,435 mg.kg^{-1} suġ.), v ktorej bola zistená najniġġia hodnota. Rovnako vysoko preukazné rozdiely boli zistené v obsahu quercetínu z lokality Liesek (6,79 mg*kg^{-1} suġ.) a Oġļ adnica (5,76 mg*kg^{-1} suġ.). Pri hodnotení obsahu kyseliny chlorogénovej ako ňalġieho významného antioxidanta sme zistili, ġe medzi vġetkými lokalitami boli ġtatistiky vysoko preukazné rozdiely ($p<0,01$), hodnoty sa pohybovali od 53,193 mg*kg^{-1} suġ. do 5,195 mg*kg^{-1} suġ. Poradie sledovaných hodnôt z jednotlivých lokalít je nasledovné: Poprad > Keġmarok > Stará ōubovŕa > Oġļ adnica > obchodná sieš> Liesek. Z hodnotenia obsahu pterostilbenu vyplýva, ġe jeho obsah bol najvyġġí vo vzorke z obchodnej siete (2,162 mg*g^{-1} suġ.) a z lokality Keġmarok (2,104 mg*g^{-1} suġ.), naopak najniġġí obsah bol vo vzorke z lokality Stará ōubovŕa (1,274 mg*g^{-1} suġ.). Medzi ostatnými hodnotami boli ġtatisticky nevýznamné rozdiely ($p>0,05$). Pterostilben je ġtrukturálne podobný s resveratrolom, je významným antioxidantom a vykazuje znaļ né antikarcinogénne a antidiabetické úļ inky. Na základe výsledkov antiradikálovej aktivity ļ uļ oriedok z vybraných lokalít Slovenska a z obchodnej siete môġeme konġtatovaš, ġe najvyġġie hodnoty boli zistené vo vzorke z lokality Keġmarok (81,18 % inhibície). Tukeyovým testom bol potvrdený ġtatisticky vysoko preukazný rozdiel medzi touto lokalitou a vġetkými ostatnými lokalitami ($p<0,01$). Z tejto lokality bol potvrdený aj najvyġġí obsah celkových polyfenolov. Kvalita testovaných vzoriek bola v súlade s platnými ġtandardnými hodnotami pre ļ uļ oriedky, ļ o podporuje myġlienku udrġateľ ného vyuġívania prírodných zdrojov v súlade s platnou legislatívou vzťahujúcou sa na chránené oblasti a ġtátne prírodné rezervácie, ochranu vodných zdrojov a pod. Hospodárskym rozvojom a moġnosťou komerļ ného vyuġitia dostupných prírodných zdrojov by bolo moġné zlepġenie ġivotnej úrovne populácie ġijúcej v týchto oblastiach.

Kľúļ ové slová: antokyaníny, antioxidaļ ná aktivita, celkové polyfenoly, ļ uļ oriedky (*Vaccinium myrtillus*, L.), kyselina chlorogénová, lokality, pterostilben, quercetin, Slovenská republika

Introduction

The fruit is associated with a protective role in health. Itś health benefits, combined with the human diet is well known and have strong support from scientific evidence. A systematic review of studies highlights positive effects on reducing risk factors for chronic diseases (Dragsted et al., 2006) and the high consumption of fruit significantly contributes to reducing the risk of cardiovascular disease (Takachi et al.,

2010), metabolic syndrome (Esmaillzadeh et al., 2006) and other diseases (Habanova et al., 2013). The fruit has high content of polyphenols and significant cardio-protective effect (Basu et al., 2010), corroborated by several epidemiological and clinical studies (Michalska, 2010, Basu et al., 2010b). Among the healthiest kinds of small fruit, the bilberry (*Vaccinium myrtillus* L.). is very rich in anthocyanins and their phenolic composition is often the subject of studies (Jaakola et al., 2002; Määttä-Riihinen et al., 2005, Burdulis et al., 2007; Lätti et al., 2008; Riihinen et al., 2008; You et al., 2011; Primetta et al., 2013). The wild bilberries (*Vaccinium myrtillus* L.) are not currently available on the market but only cultivated species of blueberries (*Vaccinium corymbosum* L.). Their composition and relative comparison was studied by Giovanelli and Buratti (2009). Despite the fact that this fruit achieves higher yields and is delicious, higher levels of antioxidant activity were recorded in bilberries from natural sources. Similar results were also published by Koca and Karadeniz (2009). The aim of this research was to monitor the biologically important substances such as total phenols, anthocyanins, quercetin, chlorogenic acid and total antioxidant activity of the bilberries from various natural localities of the Slovak Republic.

Materials and Methods

Plant material: As a biological material was ripe bilberries (*Vaccinium myrtillus* L.) sampled from five different natural localities of the Slovak Republic: Oġļ adnica 49.4375, 18.8856 (1) , Liesek 49.3607, 19.6762 (2), Poprad 49.0239, 20.28488 (3), Keģmarok 49.1385, 20.4071 (4), Stará ōubovŕa 49.3340 20.7750 (5) in the late summer of 2011(Figure 1).

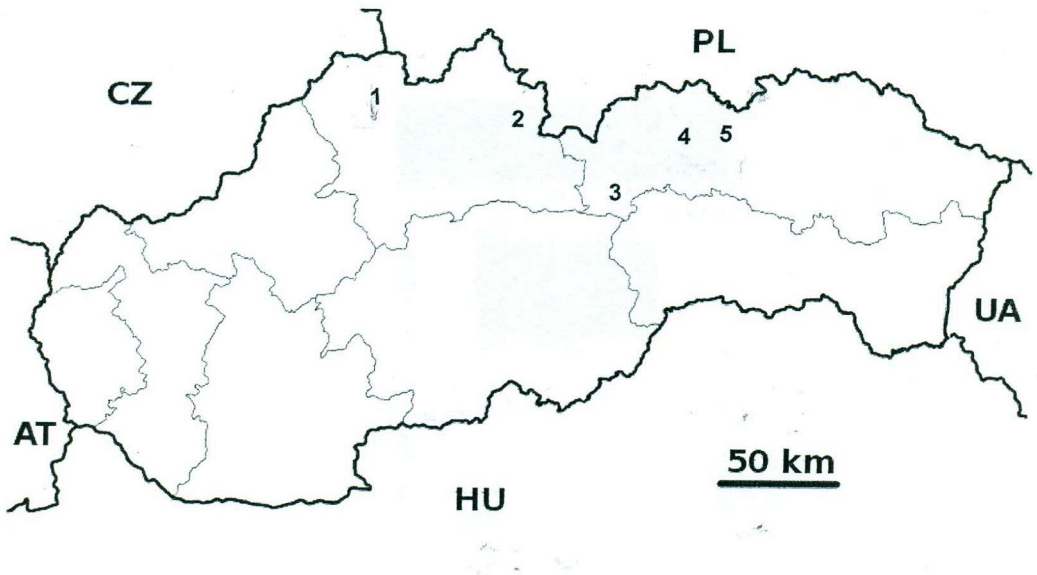

Figure 1: Map of natural localities of bilberry (*Vaccinium myrtillus* L.) in Slovak Republic: 1 ï Oġļ adnica, 2 ï Liesek, 3 ï Poprad, 4 ï Keģmarok, 5 ï Stará ōubovŕa

Obrázok 1: Mapa sledovaných prírodných lokalít brusnice ļ uļ oriedkovej (*Vaccinium myrtillus* L.) na Slovensku: 1 ï Oġļ adnica, 2 ï Liesek, 3 ï Poprad, 4 ï Keģmarok, 5 ï Stará ōubovŕa

All berries samples were kept below 8 °C after their lyophilisation to a constant dry weight. Intensification of agricultural production in the mountain and submountain areas of Slovakia in the years 1960-1989 has seen a steep decrease in natural vegetation areas of bilberry. These are currently available only at a few places such as water resources, protected areas and public nature reserves where harvesting is limited or prohibited. Any economic recovery is with the species blueberry (*Vaccinium corymbosum* L.). The current home production is insufficient ven though there is a huge interest in blueberry imports from America and Canada,which are the biggest producers on the world. Currentlythe berries may be found in the supermarkets ï the primary source of supply for the general public, it was decided to include a sample from supermarkets ï sample no. 6 ï blueberry (country of origin was Uruguay) to be compared with the content of bioactive compounds of samples from natural localities of Slovakia.

Determination of total phenolic content

The total phenolic contents were determined according to the Folin-Ciocalteu method (Waterhouse, 2002), but with some modification. A freeze-dried sample was milled in the mill. The 30 ml. tubes were 0.5 g sample and added 30 ml of extraction solvent methanol : water (80:20 v/v + 0.1 $ml*l^{-1}$ acetic acid). Allowed to stand for 1 hr then extracted in an ultrasonic bath for 15 min. Subsequently, the tubes were centrifuged at 4,000 g. Into 2.5 ml plastic tube was taken 25 μl sample from a tube, added 1.975 ml of water and 125 μl Folin-Ciocalteau reagent. Incubated max. 8 min. Then was added 375 μl 20 % Na_2CO_3 solution. Incubate 2 hours at room temperature. The absorbance was measured at 765 nm and compared with standard gallic acid.

Determination of anthocyanins content

The total anthocyanin contents was carried out as described by Giusti and Wrolstad (2001), with some modifications. Freeze-dried sample was milled in the mill. To 30 mL tubes can be 0.5 g sample and added 30 ml of extraction solvent methanol : water (80:20 w/v + 0.1 $ml*l^{-1}$ acetic acid). Allowed to stand for 1 hr then extracted in an ultrasonic bath for 15 min. Subsequently, the tubes were centrifuged at 4000 g. First, the appropriate dilution factor for the samples was determined according to the spectrophotometer response was less than 2 AU (the optimum for most spectrophotometers). Subsequently, samples we diluted a) solution of 0.025 M KCl (adjusted to pH 1.0 with hydrochloric acid) and b) solution of 0.4 M sodium acetate (adjusted to pH 4.5 with HCl). Then determined the sample absorbance at wavelengths between 520 and 700 nm separately for each solution (for pH 1.0 and for pH 4.0). The absorbance (A) of the diluted sample was then calculated as follows:

$A=(A_{520}- A_{700})_{pH1.0}$ ï $(A_{520}-A_{700})_{pH4.5}$

Then the monomeric pigment concentration in the original sample was calculated using the following formula:Antokyanins ($mg*l^{-1}$) = (A x MW x DF x 1000)*ε x 1) were MW is the molecular weight cyanidin-3-glucoside = 449.2; DF is the dilution factors, 1,000 is the factor convert g to mg and ε is the molar absorptivity (26900).

Determination of quercetin and chlorogenic acid content

The extraction of quercetin and chlorogenic acid from bilberry was adapted from the work done by Justesen and Knuthsen (2001), but with some modification. 5 g fresh

material put into 50 mL tubes, pour 10 mL 2M HCl in 50 % methanol, 10 min. ultrasound and filtered before HPLC analysis. The HPLC system consisted of a Waters system (Waters 1525, Binary HPLC Pump) 717 autoinjector, 1525 pump and 996 PDA detector. The samples were analyzed using a Purosphere C_{18} column (250x4.6; 5μm). The mobile phase consisted of 0.1% acetic acid (A) and 100 % metanol MeOH (B). The gradient was 25 ï 70 % in 0-35 min, 70 ï 85 % in 35 ï 36 min, 85 % in 40 min, 85 ï 25 % in 40 ï 41 min (B). UV spectra were recorded from 250 nm for quercetin and to 350 nm for chlorogenic acid.

Determination of antioxidant activity

The antiradical activity of the tested plants extract was measured using the stabile radical 2,2-diphenyl-1-picrylhydrazyl DPPH (Brand-Williams et al., 1995; Sánchez-Moreno et al., 1998). Freeze-dried sample was crushed in the mill. The 30 mL tubes can be 0.5 g sample and add 30 mL exktraktion solution (methanol : water, 70:20 w/v). Allowed to stand for 1 hr then extracted in an ultrasonic bath for 15 min. Subsequently, the tubes centrifuged at 4000 g. Into the cell is measured 2.5 ml of pure DPPH solution (25 mg DPPH (2,2-diphenyl-1-picrylhydrazyl)* 1.0 mL methanol) and measured the absorbance at 515 nm (A_{C0}). Then added to cells 2.44 mL of DPPH and add 62 μL sample. Violet coloration of methanolic solution of a stable radical DPPH ° (radical 2,2-diphenyl-1-picrylhydrazyl) in the presence of antioxidants demonstrated a decrease in absorbance at 515 nm after 30 minutes (A_{AT}). The anthocyans tracking efficiency added as traps radicals is calculated from the relationship: % inhibition = $[(A_{co} - A_{At})/A_{co}] \times 100$, where:

% inhibition - the ability to remove constituents of the scheduled DPPH at a time,
Aco - absorbance of control at time t = 0 min (solution of DPPH °),
A_{AT} - absorbance in the presence of an antioxidant in some of t min.

Determination of pterostilbene

The content of pterostilbene in micrograms per gram ($mg*g^{-1}$) dry weight in bilberries was performed according to Remsberg et al. (2008) with slight modifications for the conditions of liquid chromatography HPLC Waters Breeze. To the 0.5 g freeze-dried sample was added 10 mL 60 % methanol and extracted for 10 min. in an ultrasonic bath. Then followed by centrifugation at 15 000 rpm for 10 min. and injected into the HPLC. HPLC conditions: column SYMMETRY $C_{18,}$ 75 x 4.6; 3.5μm. The mobile phase consisted of 2 % acetic acid (A) and 100 % methanol MeOH (B). The gradient was 0 ï 70 % in 0-10 min, 70 ï 50 % in 10 ï 11 min (B). UV spectra were recorded at 320 nm.

Statistical analysis

All values were expressed as means ± S.D. The data were evaluated using the method of variance analysis with the Tukey test of the program Statistica Cz verzion 7.1. Difference was considered significant at $p<0.05$.

Results and Discussion

Table 1 shows the results of the analysis of the bioactive substances in bilberries (*Vaccinium myrtillus* L.) from selected natural localities of the Slovak Republic and

one sample from supermarkets (*Vaccinium corymbosum* L.). It follows that the highest value of total polyphenols was in the sample from locality Keģmarok (33772.5 ± 737.99 mg*kg^{-1} dw) and between this value and all of the other were found significant differences ($p<0.05$). The differences in content of total polyphenols between locality Stará ōubovŕa (27737.5 ± 668.00 mg*kg^{-1} dw) and Poprad (27627.5 ± 522.14 mg*kg^{-1} dw) were not statistically significant ($p>0.05$) The lowest content of total polyphenols was in a sample from the supermarket (9685.0 ± 58.63 mg*kg^{-1}dw). Vollmannová et al. (2009a) studied the total polyphenols content in selected varieties of cultivated blueberries (*Vaccinium corymbosum* L.) and indicated the values in the range from 868.3 to 2950.9 mg*kg^{-1}. Sellappan et al. (2002) evaluated the phenolic composition and antioxidant capacity of blueberries from different locallities in the State of Georgia in the United States and set compared to our results had higher content of total polyphenols (from 261.95 to 929.62 mg*100g^{-1}), which could be due to different geographical location and course of the weather, or the influence of other factors. The total anthocyanin contents was detected in a range from 5578.00 ± 420.32 mg*kg^{-1} dw in a sample from the supermarket to 2887.75 ± 114.03 mg*kg^{-1}dw from locality Keģmarok, and between these values was statistically significant difference ($p<0.05$). The second highest value of anthocyanins in a sample from Poprad (3815.25 ± 148.52 mg*kg^{-1}dw), then the locality of Stará ōubovŕa (3353.00 ± 77.80 mg*kg^{-1}dw), Liesek (3135.75 ± 123.97 mg*kg^{-1}dw) and Oscadnica (3064.75 ± 244.51 mg*kg^{-1}dw) were noted. These data are consistent with the results of Moyer et al. (2002), who evaluated the total anthocyanins, total polyphenols and antioxidant capacity of 107 genotypes *Vaccinium* L., *Rubus* L., *Ribes* L., and they point to a wide variety of fytochemical substances and antioxidant capacity in the context of the evaluated species of small fruits. Vollmannová et al. (2009b) evaluated the content of blueberries (*Vaccinium corymbosum* L.) from 615.8 to 3206.3 mg*kg^{-1} depending on the cultivar were noted. These values are compared with the values found with us below. Cho et al. (2004) indicate of the total anthocyanins content in wild bilberies ranged 1435.2 to 8227.3 mg*kg^{-1} and in garden blueberries from 1143.9 do 2415.4 mg*kg^{-1}. Kähkönen et al. (2003) reported average levels of anthocyanins in bilberries 5.997 mg*kg^{-1}. The first systematic study to detect of differences in the content and distribution of anthocanins in wild bilberries in Finland published Lätti et al. (2008). That study watched the anthocyans differences from natural sources of bilberries on the axis North-South about 1000 km in Finland. The average content of anthocyanins 2894 ± 607 mg*100g^{-1} dw was recorded, while extensive changes were recorded not only in the overall content but also in the anthocyanins in different regions (Lätti et al., 2008). The proportions of anthocyanidins and total anthocyanin contents from Turkey and Finland and results from a number of countries was reported by Primetta et al. (2013). Effects of latitude-related factors and geographical origin of anthocyanin concentration in bilberries studied other authors too (Sellappan et al., 2002; Rieger et al., 2008; Äkerström et al., 2010). The average value ± SD of quercetin in samples of bilberries (*Vaccinium myrtillus* L.) from different localities of Slovak Republic and from the supermarket is also presented in Table 1. The results indicate that the higest quercetin content was detected in sample from locality Liesek (6.79 ± 0.36 mg*kg^{-1}dw) then Stará ōubovŕa (6.03 ± 0.31 mg*kg^{-1}dw) and Keģmarok (5.83 ± 0.39 mg*kg^{-1}dw). The differences between these values were not statistically significant. Significant differences ($P<0.01$) were found in the sample from locality Poprad (5.21 ±0.29 mg*kg^{-1}dw) and from the supermarket (2.45 ± 0.07 mg*kg^{-1}dw). These results are consistent with the data published by Serafini et al. (2009).

Table 1 Total polyphenols, total anthocyanins and quercetin in bilberry (*Vaccinium myrtillus* L.) from selected natural localities of the Slovak Republic

Table 1 Celkové polyfenoly, antokyanínov a quercetinu v plodoch brusnice ļuľoriedkovej (*Vaccinium myrtillus* L.) z vybraných prírodných lokalíat Slovenskej republiky

Locality	Total polyphenols (mg*kg^{-1} dw)	Total anthocyanins (mg*kg^{-1}dw)	Quercetin (mg*kg^{-1}dw)
Oģļ adnica (1)	20752.5 ± 1006.48^{c}	3064.75 ± 244.51^{a}	5.76 ± 0.13^{a}
Liesek (2)	24095.0 ± 461.99^{d}	3135.75 ± 123.97^{a}	6.79 ± 0.36^{c}
Poprad (3)	27627.5 ± 522.14^{a}	3815.25 ± 148.52^{c}	5.21 ± 0.29^{e}
Keģmarok (4)	33772.5 ± 737.99^{e}	2887.75 ± 114.03^{a}	5.83 ± 0.39^{ab}
Stará ōubovŕa (5)	27737.5 ± 668.00^{a}	3353.00 ± 77.80^{b}	6.03 ± 0.31^{bc}
Supermarket (6)	9685.0 ±958.63^{b}	5578.00 ±420.32^{d}	2.45 ±0,07^{d}

Data are expresed as mean ± SD (for each sample n = 4), different letters in each column indicate sinificant differences at 95 % confidence level ($p<0.05$)

In the evaluation of the content of chlorogenic acid as the important antioxidant we found that among all the localities have been statistics highly conclusive differences ($p<0.01$) and content was determined in all analyzed samples ranged from 53.19 ± 1.33 mg*kg^{-1}dw to 5.20 ± 0.16 mg*kg^{-1}dw (Table 2). Higher amounts of chlorogenic acid were detected in samples harvested from locality Poprad and the order is as follows Poprad > Keģmarok > Stará ōubovŕa > Oģļ adnica > supermarket > Liesek. Moģe et al. (2011) reported an average of chlorogen acid in wild bilberries 23.1 mg*100g^{-1} and in cultivated species 70.00 mg*100g^{-1}. Chlorogen acid (5-caffeoyl-D-quinic acid - CGA) is one of the secondary metabolites of plant species (Clifford, 1999), other than similar polyfenolic substances showed health promoting effects, supported by several studies. It is an important antioxidant (Sato et al., 2011), shows a neuroprotective influence (Seung-Hwan et al., 2010) and has antimicrobial (Zhao et al., 2010), analgetic and anti-inflammatory effects as well (Dos Santos et al., 2006). Evaluation of the content of pterostilben (Table 2) indicates highest content in the sample from supermarket (2.162 ± 0.217 mg*g^{-1}) and Keģmarok (2.104 ± 0.230 mg*g^{-1}), on the contrary the minimum content was in the sample Stará ōubovŕa (1.274 ± 0.312 mg*.g^{-1}). Among other values were not statistically significant. ($p>0.05$). Pterostilbene is structurally similar to resveratrol and shows a considerable anticarcinogene and antidiabetic effects and is an important antioxidant (Rimando et al., 2002). On the basis of the results of the antioxidant activity of bilberries from selected localities of Slovakia plus the sample from the supermarket, it may be concluded that the highest values were found in a sample from Keģmarok (81.180 ± 2.020 % inhibition).

Table 2 The content of chlorogenic acid, pterostilbene and antiradical activity of bilberry (*Vaccinium myrtillus* L.) from selected natural localities of the Slovak Republic

Table 2 Obsah kyseliny chlorogénovej, pterostilbenu a antioxidaļ ná aktivita v plodoch brusnice ļ uļ oriedkovej (*Vaccinium myrtillus* L.) z vybraných lokalít Slovenskej republiky

Locality	Chlorogenic acid (mg*100g^{-1})	Pterostilben (mg*g^{-1})	Antioxidant activity (% inhibition DPPH)
Oġļ adnica (1)	25.86 ± 0.48^{c}	1.905 ± 0.236^{ab}	76.937 ± 1.474^{b}
Liesek (2)	5.20 ± 0.16^{a}	2.001 ± 0.121^{ab}	75.232 ± 0.638^{ab}
Poprad (3)	53.19 ± 1.33^{f}	1.601 ± 0.185^{bc}	72.950 ± 0.716^{a}
Keġmarok (4)	49.70 ± 1,68^{e}	2.104 ± 0.230^{a}	81.180 ± 2.020^{c}
Stará ōubovŕa (5)	38.48 ± 1.30^{d}	1.274 ± 0.312^{c}	72.837 ± 1.095^{a}
Supermarket (6)	8.91 ± 0.13^{b}	2.162 ± 0.217^{a}	75.742 ± 0.545^{ab}

Data are expresed as mean ± SD (for each sample n = 4), different letters in each column indicate significant differences at 95 % confidence level ($p<0,05$)

Applying the method of variance analysis with the Tukey test it was confirmed that there was a statistically highly significant difference between this locality and any other localities ($p<0.01$). Kalt et al. (2000) found a positive correlation relationship between antioxidant capacity and the concentration of total polyphenols. This finding agrees with data previously reported by other authors (Kaļ ániová et al., 2008; Fatrcová-Ġramková et al., 2013).

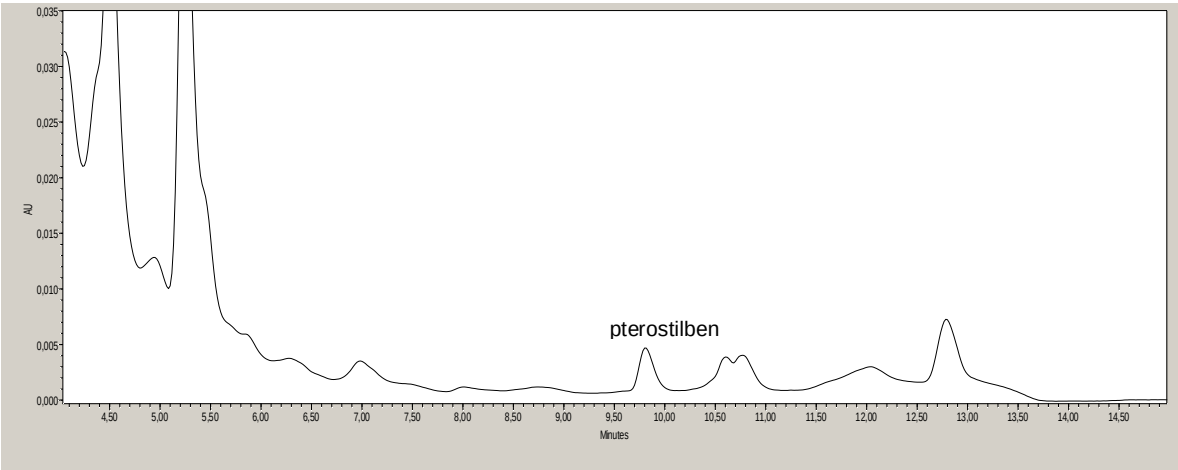

Figure 2: Chromatogram for pterostilbene

Obrázok 2: Chromatogram pre pterostilben

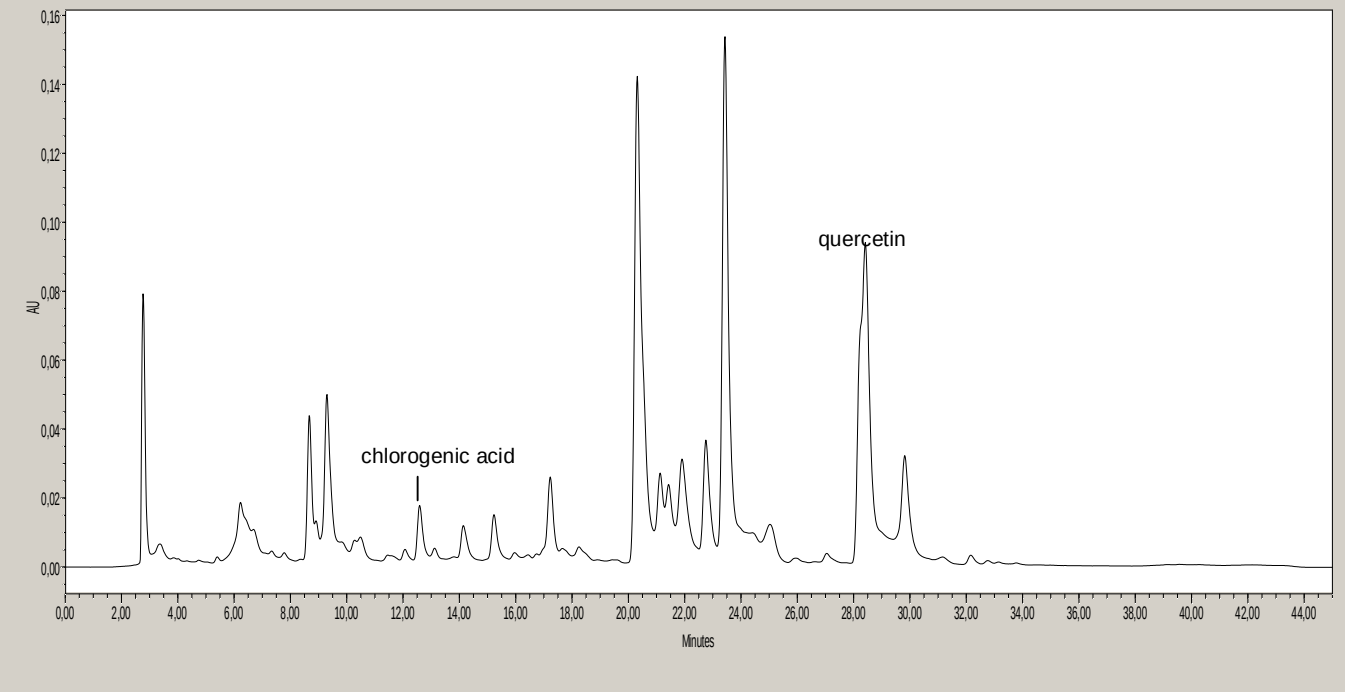

Figure 3: Chromatogram for chlorogenic acid and quercetin

Obrázok 3: Chromatogram pre quercetin a kyselinu chlorogénovú

Beliveau and Gingras (2005) reported that the antioxidant properties are a common feature of many plants and actually impede the action of free radicals, in particular in the oxidation of the blood vessel walls, causing a number of vascular diseases. However, it is necessary to affirm the limit of this theory and focus on food as a source of antioxidants.

Conclusion

This study offered evidence of the impact of geographical variability in the Slovak Republic on selected quality indicators of wild bilberry (*Vaccinium myrtillus* L.). The content of total polyphenols range from 9685.0 to 33772.5 mg*kg^{-1} dw in evaluated samples and this difference was significant in relation to the locality. Similarly, by other bioactive indicators, which have been tracked (anthocyanins, chlorogenic acid, quercetin, pterostilben and antioxidant activity) was confirmed significant relationship to the locality. Influece of other factor such as the weather, the degree of ripeness, method of collection and others is also expected. The quality of tested samples was in accordance with the standard values for the bilberries, which makes it possible to support the focus on the sustainable use of natural resources in accordance with current policy efforts applicable to protected areas and public nature reserves, protection of water resources, etc.

Acknowledges

The Paper was supported by the project: Development of International Cooperation for the Purpose of the Transfer and Implementation of Research and Development in Educational Programs conducted by the Operational Program: Education, ITMS code: 26110230085 and by VEGA project No 1/0544/13 òThe research of agrienvironmental indicators of sustainability and production capability of agroecosystem by diversification of crop rotation pattern in changing climateò

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