Published in Journal of Food Composition and Analysis, 2018, vol. 66, pp. 81-89, which should be cited to refer to this work.

DOI: https://doi.org/10.1016/j.jfca.2017.12.006

Original Research Article

Bioactive compounds and antioxidant capacity of *Lonicera caerulea* berries: comparison of seven cultivars over three harvesting years

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Running title: Bioactives of seven cultivars of Lonicera berries

Abstract

This study aimed at characterization of bioactive compounds of haskap berries (*Lonicera caerulea* L.) cultivated in Switzerland and their antioxidant capacity. Seven cultivars *i.e.* 'Berry Smart Blue', 'Indigo Gem', 'Indigo Treat', 'Morena', 'Tundra', 'Uspiech' and 'Viola' were compared over three harvesting years. Cyanidin-3-glucoside was the main anthocyanin found in haskap berries and at the same time the predominant phenolic compound identified, its content varied between 12.5 and 87.5 mg/g of dry matter. 'Morena', 'Indigo Treat' and 'Uspiech' cultivars emerged as rich in total polyphenols, total anthocyanins and antioxidant capacity. The content of individual polyphenols was the highest in 'Indigo Treat' berries. Ascorbic acid content was in the range of 1.78 - 4.21 mg/g DM. It was the highest in 'Indigo Gem' and 'Indigo Treat' cultivars. Sugars were more concentrated in 'Indigo Gem', 'Indigo Treat' and 'Viola'. The significant differences in bioactive content between different harvesting years evoked by different weather conditions were noted. 'Indigo Treat' cultivar deserves further attention as potentially well adapted for further cultivation in Switzerland and providing berries rich in bioactive compounds, which might be of interest to the consumers concerned about health promoting properties of their diet.

Keywords: food analysis, food composition, honeysuckle, *Lonicera caerulea*, antioxidant activity, polyphenols, anthocyanins, HPLC, fruits

1. Introduction

Results of numerous studies support the recommendation of World Health Organisation to increase the consumption of fruit and vegetables (WHO, 2003). It is recommended to consume a minimum of 400 g of fruit and vegetables daily. High intake of fruits and vegetables (5-7 portions per day) reduces the all-cause mortality (Hjartåker et al., 2015; Nguyen et al., 2016; Oyebode et al., 2014). Berries are low in calories and rich in fibre. They contain ascorbic acid, vitamin E and are abundant in polyphenols. The large proportions of berry polyphenols constitute anthocyanins (Del Bo' et al., 2015). Increasing number of evidences confirm that high anthocyanins intake is associated with decreased risk of cardiovascular diseases (Cassidy et al., 2016, 2013). Apart from growing of berries well established in Switzerland *i.e.* strawberries or raspberries, local producers get interested in establishing the cultivation of berries which are not typical for that region such as goji (Kosińska-Cagnazzo et al., 2017), aronia ("Passion for berries," 2016) or haskap berries.

Lonicera caerulea L. plant belonging to the *Caprifoliaceae* family is bearing haskap berries, also called honeysuckle berries. The plant originates from high mountains or low-lying wet areas in northern Russia, China and Japan (Rupasinghe, 2012). It is very hardy and can resist to temperatures up to -40 °C, while its flowers resist up to -7 °C. Its fruits are purple berries, about 1-2 cm long and 1 cm wide. In Japan haskap berries are used in traditional medicine and recognized for slowing the aging process, preventing heart diseases and gastrointestinal dysfunction (Celli et al., 2014). The early ripening of haskap berries which takes place between May and June, as early as strawberries and before all other fruits, might be its great advantage. The high content of bioactive compounds such as ascorbic acid and polyphenols including anthocyanins in haskap berries were reported in recent studies (Rupasinghe, 2012). Anthocyanins are widely distributed plant pigments, responsible for red to blue colour of fruits and flowers. Structurally anthocyanins are composed of anthocyanidin and sugar, bound through a glycoside bond. The most common anthocyanidins are petunidin, cyanidin, pelargonidin, delphinidin, malvidin and peonidin appearing in plants bound to glucose, galactose, arabinose, rhamnose or xylose. Due to its high content of cyanidin-3-glucoside the haskap berries might have antioxidant, anti-inflammatory, antimicrobial, cardioprotective and hepatoprotective activities (Lila et al., 2016; Myjavcová et al., 2010; Wu et al., 2015).

The studies on bioactivity of haskap berries started only recently, therefore the results are still scarce. Some reports from both *in vivo* animal studies and *in vitro* cell line investigations showed that haskap berries may protect from inflammation and oxidation triggered diseases (Rupasinghe et al., 2015). Blue honeysuckle extract in rat model attenuated adjuvant-induced arthritis symptoms with crosstalk of anti-inflammatory and antioxidant effects (Wu et al., 2015). The application of *L. caerulea* berry extract protected liver against the damage caused by lipopolysaccharide which shows their potential in prevention of hepatitis (Wang et al., 2016a). Within 30 different fruits tested ranging from apples, bananas, pineapples, grapes, orange to numerous types of berries, honeysuckle berries exhibited the highest inhibitory activity towards carbohydrate degrading enzymes indicating its possible utility in preventing obesity and type-2 diabetes (Podsędek et al., 2014). According to our knowledge only one human study on haskap berries was carried out so far. The results showed that the metabolites of bioactives can be found in blood and urine of healthy volunteers after haskap berries consumption (Heinrich et al., 2013).

Plantations of haskap berries are well established in Russia and Japan (Caprioli et al., 2016; Lefèvre et al., 2011) whereas they are relatively unknown in Europe and North America. They continuously gain interest in Poland (Kusznierewicz et al., 2012; Wojdyło et al., 2013), Czech Republic (Jurikova et al., 2012), China (Wang et al., 2016), the USA (Chaovanalikit et al.,

2004) and Canada (Celli et al., 2015). To our knowledge this is the very first report on haskap berries from Switzerland. It is well known that the profile and content of bioactive compound are highly depend on numerous factors such as genotype, climatic conditions or agronomic practices. The objective of this study was to quantify the major free sugars, ascorbic acid, phenolic compounds as well as to evaluate antioxidant activity of seven cultivars of haskap berries grown in Switzerland and harvested over a 3-year period. Based on the results obtained, the *Lonicera* cultivar well-suited for commercial plantation in Switzerland will be selected.

2. Materials and methods

2.1 Reagents

Folin-Ciocalteu's phenol reagent (FCR), potassium phosphate monobasic, chlorogenic acid, ferulic acid, *p*-coumaric acid, gallic acid, caffeic acid, quercetin-3-rutinoside, quercetin-3-β-D-glucoside, p-hydroxybenzoic acid, vanilic acid, salicylic acid, syringic acid, sinapic acid, protocatechuic acid, gentisic acid, tyrosol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, sulfuric acid, hydrochloric acid, sodium chloride, sodium phosphate, glucose, fructose and sucrose were supplied by Sigma-Aldrich (Buchs, Switzerland). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was provided by Roche (Basel, Switzerland). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, potassium chloride and acetic acid were supplied by Acros Organics (Thermo Fisher Scientific, Geel, Belgium). Sodium acetate and oxalic acid were purchased from Riedel-de Haën (Seelze, Germany). Formic acid was obtained from Merck (Darmstadt, Germany). Ethanol was supplied by Cochimy (Martigny, Switzerland). Acetonitrile was provided by Macron Fine Chemicals (Center Valley, PA, USA). Cyanidin-3-glucoside and catechin were obtained from Extrasynthese (Genay, France).

Deionized water was obtained using Milli-Q purification system (Millipore AG, Zug, Switzerland).

2.2 Plant material

Seven cultivars of *Lonicera caerulea* var. *kamtschatica i.e.* 'Berry Smart™ Blue', 'Indigo Gem', 'Indigo Treat', 'Morena', 'Tundra', 'Uspiech' and 'Viola' were included in the study. Approximately 20 cm-high plants were obtained from Polish grower Gospodarstwo Ogrodnicze Tadeusz Kusibab (www.in-vitro.pl) where they were developed on the licence of the University of Saskatchewan (Canada). It was assumed that these cultivars might be adapted to climatic conditions in Switzerland. The plants were planted in Savièse (Valais, Switzerland, 46°25’’N 7°35’’E and 900 m of altitude) in 2013. The first berries were harvested in 2014. The bushes were about 20-50 cm high. The berries were harvested from the end of May to the middle of June in three consecutive years (2014-2016). Only fruits at commercial maturity stage, based on the colour and texture were collected (Fig. 1). For each cultivar between 30 and 50 g of fruits were obtained. Immediately after harvest, fruits were placed in plastic bags and stored at -20 °C until analysed. Stored samples were thawed at room temperature.

2.3 Climatic conditions

The meteorological data were provided by the Federal Office of Meteorology and Climatology MeteoSwiss. The data were recorded at the weather station nearest to the growing fields during three harvesting years. Mean temperature, sun exposure and total precipitation from February to June in the years 2014, 2015 and 2016 are presented in Table 1.

2.4 Water content, total soluble solid (TSS) and pH

Water content was measured with a halogen moisture analyser (HG53, Mettler-Toledo, Greifensee, Switzerland) in order to express the results on dry matter (DM) basis. Total soluble solid (TSS) content was measured in juice from pressed berries with a refractometer (ATAGO PR-1, Kunzmann Gossau-Zurich, Switzerland). Results were expressed in °Brix. pH of berries was measured in juice from pressed berries with a pH-meter (827 pH-Lab, Metrohm, Zofingen, Switzerland).

2.5 Free sugars and ascorbic acid content

About 7 g of berries were immersed and crushed in oxalic acid solution (10 g/L) with one drop of sunflower oil to prevent foaming (Gastro 200 blender, Bamix, Mettlen, Switzerland). The mixture was extracted in an ultrasonic bath (working frequency 35 khz, VWR, Dietikon, Switzerland) for 20 min. The extraction with oxalic acid solution was carried out in triplicate for each berry cultivar. The volume was adjusted to 100 mL with oxalic acid solution. This extract was centrifuged at 900 g for 10 min (Hettich Universal 1200, Bäch, Switzerland). The supernatant was used to measure sugars, ascorbic and organic acids content by HPLC as described previously (Kosińska-Cagnazzo et al., 2017). An Agilent 1220 Infinity series liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) was composed of an autoinjector, a binary pump, a diode-array detector (UV-DAD) and a refractive index detector (RID). A 5 µL-sample was injected onto an Aminex HPX-87H column (300 mm × 7.8 mm i.d., particle size of 5 µm, Bio-Rad, Hercules, CA, USA) protected by a Micro-Guard cartridge (Bio-Rad). The mobile phase was composed of 0.05 mmol/L sulfuric acid and was delivered in an isocratic mode at a flow rate of 0.5 mL/min. The column temperature was kept at 35 °C. Each sample was filtered through 0.45 µm Exapure PTFE filter (Alys Technologies, Bussigny-près-Lausanne, Switzerland) before injection and analysed in three different vials. Ascorbic acid detection was performed with the DAD at 254 nm, whereas sugars were detected with the RID.

2.6 Phenolic compound profile analysis by HPLC

About 2 g of crushed berries were extracted with a solution of ethanol acidified with 1% of formic acid. The extraction was carried out during 20 min in an ultrasonic bath. The sample was centrifuged at 1390 g for 10 min (MF 20-R, rotor AMF 20-8, Awel, Blain, France) and the supernatant was filtered (LS 14 ½ filters, Schleicher & Schüll, Feldbach, Switzerland). The residue was extracted twice more, the supernatants were combined and adjusted to a final volume of 50 mL. Extraction was carried out in triplicate for each berry sample. The liquid extracts were stored frozen at -20 °C until analysed The quantification of selected anthocyanins and polyphenols was performed by HPLC and total anthocyanins content and antioxidant capacity using spectrophotometric methods.

For HPLC analysis the samples were diluted if needed with 0.1% formic acid before measurements. A portion of 1 µL of the sample was injected onto a Kinetex EVO column (100 Å, 100 mm × 2.1 mm, particle size of 2.6 µm, Phenomenex, Torrance, CA, USA). The separation was performed as previously described (Kosińska et al., 2013). The mobile phase was delivered at a constant flow rate of 0.3 mL/min in a gradient mode. The mobile phase A consisted of 1% aqueous formic acid and B of 1% formic acid in acetonitrile (v/v). The separation started isocratically with 100% of A for 2 min, then the following linear gradient was employed: 0-10% B from 2 to 25 min, kept at 10% B until 26 min, from 10 to 60% B until 30 min and kept at 60% B until 35 min. Detection was carried out at 260, 280, 320, 340 and 500 nm. Column temperature was set at 40 °C. Chlorogenic and ferulic acids were quantified at 320 nm, rutin at 340 nm and the cyanidin-3-glucoside at 500 nm.

2.7 Total anthocyanins content (TAC)

Total monomeric anthocyanins content was determined by differential pH method (Giusti and Wrolstad, 2001). Aliquots of 20 μL of berry extracts were placed into microplate wells in duplicates. 280 μL of 0.4 mmol/L sodium acetate buffer (pH 4.5) or of 0.025 mmol/L potassium chloride solution (pH 1.0) was added. Absorbance was measured at 508 nm and 700 nm using Infinite M200 Pro microplate reader (Tecan, Männedorf, Switzerland).

$$content\ of\ anthocyanins\ (mg/g) = \frac{A \times MW \times DF \times V \times 1000}{\varepsilon \times l \times m}$$

where

$A = (A_{508nm}-A_{700nm})pH\ 1.0 - (A_{508nm}-A_{700nm})pH\ 4.5$

MW - molecular weight = 449.4 g/mol for cyanidin-3-glucoside

DF - dilution factor

V - extraction volume

ε – molar absorption coefficient = 26 900 L × mol^{-1} × cm^{-1} for cyanidin-3-glucoside

l – optical path length in cm

m – berry sample weight

Results were given in mg of cyanidin-3-glucoside equivalents per g of berry DM.

2.8 Antioxidant capacity

2.8.1 Folin-Ciocalteu reagent (FCR) reducing capacity assay

The reducing capacity was determined with FCR method (Singleton et al., 1999) with modifications of Horszwald and Andlauer (Horszwald and Andlauer, 2011). The method is traditionally described as total phenolic content however due to its lack of specificity it reacts with all reducing components present in the sample (Huang et al., 2005). The standard solutions of gallic acid at different concentration were prepared in milli-Q water. Aliquots of 25 μL of blanks, standards and samples were pipetted into microplate wells. The plate was placed in the Infinite M200 Pro microplate reader and 250 μL of diluted FCR (1+14 (v/v) in milli-Q water) was added by automatic injector. After 10 min of reaction in the dark at room temperature, 25 μL of 5% aqueous Na_2CO_3 were added to each well. After an incubation of 20 min the absorbance at 755 nm was measured. Results were expressed as gallic acid equivalents per g of berry DM.

2.8.2 DPPH assay

Antioxidant capacity of berries was assessed using DPPH assay as described by Brand-Williams et al. with some modifications (Brand-Williams et al., 1995). Briefly, aliquots of 25 µL of samples, Trolox standards and blanks in ethanol were placed into microplate wells and 300 µL of DPPH solution (0.4 mg/L in ethanol) was added by an Infinite M200 Pro microplate reader automatic injector. After 30 min of reaction in the dark, absorbance was measured at 517 nm. Results were expressed in μmol of Trolox equivalents (TE) per g of berry DM.

2.8.3 Trolox equivalent antioxidant capacity (TEAC) by ABTS assay

TEAC was assessed by measuring scavenging activity towards $ABTS^{\bullet+}$ according to Re et al. (Re et al., 1999) with the modifications of Horszwald and Andlauer (Horszwald and Andlauer, 2011). Briefly, $ABTS^{\bullet+}$ solution was prepared by reacting 2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt at a concentration of 7 mmol/L with 2.45 mmol/L potassium persulfate at room temperature for 16 h. The solution obtained was then diluted at 1:50 ratio (v/v) with ethanol to an absorbance of 0.70 at 734 nm. Aliquots of 20 µL of blanks, Trolox standards and samples appropriately diluted in ethanol were pipetted into microplate. 290 µL of radical solution was added in each well by automatic injector. After

6 min of the reaction time taking place at 30 °C in dark, the absorbance was measured at 734 nm. Results were expressed in μmol of TE per g of berry DM.

2.9 Statistical analysis

All measurements were done in triplicate, except when indicated. The final results were given as mean and standard deviation. One-way ANOVA with Tukey's multiple comparisons test were performed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA) to test for significant differences. The differences were considered significant at p-value $<$ 0.05. Firstly, all cultivars were compared within each year. Secondly, each cultivar was individually compared over the three harvesting years.

3 Results and discussion

3.1 Water content, total soluble solids (TSS) and pH

Water content of the berries ranged from 76 to 85% (Table 2). On general, the highest values were noted for 'Morena' and 'Tundra' cultivars and the lowest for 'Indigo Gem'. Only for fruits harvested in 2016 no significant differences in water content between cultivars were noted. The water content varied between harvest years. The highest water content was noticed in 2016.

The Brix value reflects the TSS content in fruits. The TSS content in haskap berries studied ranged from 9.7 to 18.3 °Brix. On average, 'Indigo Gem' and 'Indigo Treat' had the highest TSS content amounting to 15.3 and 14.0, respectively, whereas 'Tundra' and 'Morena' had the lowest TSS content. Average TSS values for 2016 (10.8 °Brix) were clearly lower than those noted for 2014 and 2015, *i.e.* 14.8 and 14.6, respectively. Wojdyło et al. reported that TSS in haskap berries grown in Poland ranged from 10.1-15.8 °Brix (Wojdyło et al., 2013). Similar results were also noted for blueberries and blackcurrant (Camps et al., 2010; Gündüz et al., 2015).

pH measured in freshly pressed berries was around 3.0 for all berries. On average, the pH was the lowest in 2016 (2.9) and in 2014, it was the highest (3.2). 'Morena' seems to be the cultivar with the more acidic pH, whereas 'Indigo Treat' was the least acidic.

3.2 Free sugar and ascorbic acid content

Free sugar content is an important parameter determining taste of berries and therefore consumer acceptance. Fructose and glucose were predominant free sugars in haskap berries (Table 3). Significant differences in sugar content between cultivars were noted. Glucose content in berries ranged between 80.0 and 327.3 mg/g DM. Fructose content was higher, with a range of 140.6 - 337.4 mg/g DM. The berries of 'Indigo Gem', 'Indigo Treat', 'Berry Smart Blue' and 'Viola' cultivars were richest in free sugars. However, the results varied considerably over the three year harvest. On average, the highest glucose and fructose content was in 2015 and the lowest in 2014. The concentration of sugars in berries is largely affected by the ripeness stage, genotype and climatic conditions (Kosińska-Cagnazzo et al., 2017; Pokorna-Jurikova and Matuskovic, 2007). The weather in 2014 was characterised by high average temperatures and sunshine time but very low total precipitation in May and June. In general high exposition to light increase sugar content in berries, however the results vary for different species (Zheng et al., 2009). Large differences in fructose and glucose content between different *L. carulea* genotypes was noted also by other authors (Rupasinghe et al., 2015; Wojdyło et al., 2013). Content of sugars in berries is an important parameter influencing the sensory properties and consumer acceptance of berries. In comparison with other fruits like blackberries, strawberries and raspberries, analysed berries contain similar amount of sugars i.e. 3.2 – 8 g/portion ("USDA National Nutrient Database for Standard Reference : USDA ARS," n.d.). Relatively higher content of sugars might have positive effect on the sensory quality of haskap berries regarding the possibility to mask their typical astringency. It might be supposed that cultivars with higher sugar content would gain higher consumer acceptance.

The content of ascorbic acid in haskap berries cultivars studied is presented in the Fig. 2. Ascorbic acid content was in the range of 1.78 - 4.21 mg/g DM. It was the highest in 'Indigo Gem' and 'Indigo Treat' cultivars. Similar values were reported for *Lonicera* genotypes grown in Poland and Russia (Caprioli et al., 2016; Ochmian et al., 2012; Skupien et al., 2007). In comparison with other berries, haskap berries are rich in ascorbic acid, its content is higher than for blueberry, raspberry, aronia or strawberry but lower than in blackcurrant (Celli et al., 2014; Gopalan et al., 2012; Rupasinghe, 2012). The values of Population Reference Intake for vitamin C are set at 110 and 95 mg/day for adult men and women respectively (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2013). The food product is considered rich in specific nutrient when one portion provides 20% of recommended daily intake. An 80-g portion of haskap berries provides between 24 and 80 mg of ascorbic acid, therefore the berries of all cultivars analysed are rich in ascorbic acid.

3.3 Phenolic compound profile

Phenolic compounds were identified in haskap berry extracts by the comparison of retention time and UV-Vis spectra with standard compounds. Four phenolic compounds such as cyanidin-3-glucoside, chlorogenic acid, ferulic acid and rutin were identified and quantified (Table 4). Cyanidin-3-glucoside was the main anthocyanin found in haskap berries and at the same time the predominant phenolic compound. It is a red-violet pigment, found in berries such as aronia or blueberries, as well as in dark grapes (Del Bo' et al., 2015). Cyanidin-3-glucoside content varied between 12.5 and 87.5 mg/g DM. This finding is in accordance with the literature data reporting that cyanidin-3-glucoside accounts to 80-90% of the total anthocyanins in haskap berries (Caprioli et al., 2016; Chaovanalikit et al., 2004; Jurikova et al., 2012; Ochmian et al., 2012). 'Indigo Treat' was richest in that compound whereas 'Berry Smart Blue' had the lowest content. On average, the content was significantly higher in 2014 than in two other harvest years. Higher growth temperatures causes more rapid development of fruit colour than that at lower growth temperatures (Wang and Zheng, 2001). It was also reported by Parr and Bowel that UV radiation is needed for development of anthocyanin pigments (Parr and Bolwell, 2000). Year 2014 was not only characterised by high sunshine time but also by considerably lower total precipitation. The studies have shown increase in anthocyanins accumulation due to water deficit (Stefanelli et al., 2010). In comparison with other types of berries, haskap berries have very high content of anthocyanins (Chen et al., 2014).

The second most concentrated phenolic compound identified in the analysed haskap berries was chlorogenic acid. It is a common polyphenol produced by plants, mainly found in coffee, apples, pears, eggplant and berries (Crozier et al., 2009). Structurally, it is an ester of caffeic acid and quinic acid. Its content in the berries analysed amounted to 2.3 - 10.1 mg/g DM. Concerning the individual berry cultivars 'Uspiech' and 'Indigo Treat' were richest in chlorogenic acid. In general, its content was higher in 2014 than in other harvest years. Quite wide ranges between 0.1 mg/g DM (Zadernowski et al., 2005) and 8.6 mg/g DM (Jurikova et al., 2012) were reported for this compound in the literature data. Higher content of chlorogenic acid comparing to the results of the present study was noted for different haskap cultivars grown in Canada with amounts varying between 201–234 mg/100 g FW. Ferulic acid is a ubiquitous compound in plant tissues, used in the synthesis of lignin, which compose the plant cell walls. It is commonly found in grains, citrus, coffee, bamboo shoots, and in smaller quantities in berries (Zadernowski et al., 2005). Its content in haskap berries analysed was in the range of 0.1 - 1.1 mg/g DM. Literature values were lower and ranged from 0.037 mg/g DM (Zadernowski et al., 2005) to 0.16 mg/g DM (Jurikova et al., 2012). The content of rutin (0.9 and 3.7 mg/g DM) was in accordance with the literature data for haskap berries ranging from

0.8 mg/g DM (Skupien et al., 2007) to 5 mg/g DM (Jurikova et al., 2012). Also rutin content was highest in 2015 for all varieties.

3.4 Total monomeric anthocyanin content

TAC is presented in Fig. 3. The results obtained range between 8.4 and 41.1 mg of cyanidin-3-glucoside eq./g DM, whereas literature refers to values between 14.3 and 65 mg of cyanidin-3-glucoside eq./g DM for haskap berries (Celli et al., 2015, 2014; Kusznierewicz et al., 2012; Rop et al., 2011; Rupasinghe, 2012). Also in this assay 'Indigo Treat' had highest content of analysed compounds. It is worth mentioning that the results were the highest in 2014 and similar in 2015-2016.

3.5 Antioxidant capacity

An assay with FCR, traditionally called total phenolic content is based on an electron transfer reaction during which the reducing capacity of tested extract is evaluated (Huang et al., 2005). The FCR reducing capacity of tested berries was in the range of 12.6 - 42.3 mg GAE/g DM (Fig. 3), in accordance with the values cited in the latest review *i.e.* 7.0 to 57.1 mg GAE/g DM (Celli et al., 2014). The results showed that 'Morena', 'Indigo Treat' and 'Uspiech' were the cultivars with the highest FCR reducing capacity. Antioxidant capacity of the sample was also estimated with DPPH and ABTS assays. In its radical form $DPPH^{\bullet}$ absorbs at 517 nm, in the presence of antioxidant it undergoes reduction and the absorption disappears. The measurement of the decrease in the absorbance allows estimation of the antioxidant activity of the sample. Similarly, the antioxidants present in the sample scavenge ABTS radical resulting in decrease of blue colour. ABTS results were comprised between 125.3 and 485.0 μmol TE/g DM (Table 5). Results were significantly higher in 2014. Literature reported ABTS values were from 130 to 500 μmol TE/g DM (Kusznierewicz et al., 2012; Wojdyło et al., 2013; Zhao et al., 2012).

DPPH assay showed antioxidant capacities in the range of 60.0 - 228.4 µmol TE/g DM. On average results from this study were thus similar to previous studies. In these three assays, antioxidant capacities were maximal in 'Morena' cultivar, followed by 'Indigo Treat' and 'Uspiech' cultivars. 'Berry Smart Blue' was always the cultivar with lowest antioxidant capacity.

The selection of cultivar well suited to be grown in Switzerland should not be based only on best yield and sensorial quality. The consumers are more and more concerned about health promoting properties of their food. In order to guide potential haskap berry growers to select the type of cultivar which contains optimal bioactive phytochemicals, we evaluated fruits of different cultivars grown under specific conditions. The analysis of berries of different harvest years enabled to show the effect of weather conditions on the bioactive content.

4 Conclusions

Seven cultivars of *Lonicera caerulea* berries were tested for their bioactive content and antioxidant capacity over three harvesting years. This preliminary study showed that 'Indigo Treat' cultivar deserves further attention as potentially well adapted for cultivation in Switzerland. It had the highest contents of ascorbic acid and polyphenols, its antioxidant capacity is also higher than those of other cultivars. Furthermore, it might gain the consumer acceptance due to higher sugar content than other cultivars analysed, which probably masks the typical astringent taste of haskap berries. Also 'Indigo Gem' and 'Morena' cultivars should be further investigated as rich in ascorbic acid, sugars, and antioxidant capacity, respectively. Finally, this study showed that haskap berries are very interesting for their bioactive content, globally higher than in other commonly consumed berries. Sensory analysis should be carried out in order to determine consumer preferences.

5 Acknowledgements

We would like to express our gratitude to Nadine Lacroix for providing us with haskap fruits as well as to Agroscope Research Centre for supervision of fruits cultivation. The partial financial support for this study from Swiss Food Research is also greatly acknowledged.

Conflict of interests

The authors declare no conflict of interests.

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Figure captions

Figure 1 Appearance of seven haskap berries cultivars harvested in 2016.

Figure 2 Content of ascorbic acid in seven haskap berries cultivars harvested over three years. Each bar represents mean ± standard deviation of triplicate analysis. Means with different letters within one harvesting year are significantly different ($p \leq 0.05$).

Figure 3 Folin-Ciocalteau reducing capacity (FR-RC) expressed as gallic acid equivalents and total anthocyanins content (TAC) expressed as cyanidin-3-glucoside equivalents of seven haskap berries cultivars harvested over three years. Each bar represents mean ± standard deviation of triplicate samples. Means with different letters within one harvesting year are significantly different ($p \leq 0.05$).