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Original Article

Physicochemical and Bioactive Characteristics of Wild Grown Bilberry (*Vaccinium myrtillus* L.) Genotypes from Northeastern Turkey

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Abstract

In the present study, fruit weight, fruit firmness, external fruit color, soluble solid content, pH, titratable acidity, vitamin C, total phenolics, total anthocyanins and antioxidant capacity of eight wild bilberry grown in Coruh valley in northeastern Turkey were determined. The blueberry cv. 'Bluecrop' was also studied to make comparison with bilberry genotypes. Antioxidant activity of bilberry and blueberry fruits were comparatively assessed by FRAP (Ferric reducing antioxidant power) assay. Significantly higher contents of phenolics and anthocyanins were found in bilberry fruits comparted with blueberry fruits. However, blueberry cv. 'Bluecrop' exhibited higher fruit weight and vitamin C content than bilberry fruits. Total phenolic and total anthocyanin content was 327 mg gallic acid equivalent and 142 mg of cyanidin-3-glucoside equivalent in 100 g fresh fruit in cv. 'Bluecrop', while it was between 492-563 mg gallic acid equaivalent and 307-342 mg of cyanidin-3-glucoside equivalent in 100 g fresh fruits of bilberry accessions. Moreover, wild accessions had approximately 2-3 folds higher antioxidant capacity than cv. 'Bluecrop'. The obtained results suggest the possibility of improving the bioactive and antioxidant properties of bilberry cultivars by using wild ones in cross breedings. It was also concluded that genotypes significantly affect the bioactive content of fruits and consequently the possibility of using wild bilberry fruits as a potential source of natural antioxidants in food industry is worth to be considered.

Keywords: bilberry, composition, pomology, diversity

Introduction

The increased concern of consumers towards food products rich in nutraceuticals has led to an increased interest in the research about the bioactive compounds in fruits (Canan *et al*., 2016; Yazici and Sahin, 2016). In this respect, the berries, in particular wildly grown, are an important fruits group, that is mainly preferred for their specific sweet to sour taste, aroma and colourful; these fruits are investigated not only for their nutritional properties, but also for their bioactive constituents (Veberic *et al*., 2009; Paredes-Lopez *et al*., 2010; Landete, 2012; Milivojevic *et al*. 2012).

Considering the genetic differences among wild relatives and natural population or cultivars of berries, a potential variability can be observed in their physicochemical and bioactive contents (Remberg *et al*., 2007; Mikulic-Petkovsek *et al*., 2012; Milivojevic *et al*., 2013).

The bilberry (*Vaccinium myrtillus*) is a low-growing shrub native to northern Europe, but is now also found in parts of North America and Asia. Bilberry is also known as European blueberry, whortleberry, huckleberry and blaeberry. It belongs to a large genus (*Vaccinium*) of plants that also contain blueberry (*Vaccinium corymbosum*) and cranberry (*Vaccinium macrocarpon*). Bilberries are sometimes also called as blueberries because both have similar appearance and are close relatives, but the true blueberry is native to the United States (Valentova *et al*., 2006). Bilberry usually grows in heaths, meadows and moist coniferous forests, and its growth is favoured by moderate shade and moderately humid ground conditions. The bilberry shrubs have small (5-9 mm in diameter) fruit, bluish black in colour, with many seeds (Riihinen *et al*., 2008).

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Bilberry is sold as fresh, frozen and dried whole berries, as well as in the form of preserves, jams, or juices, and, increasingly, liquid or powdered concentrates are sold as food supplements. Bilberry contains a variety of phenolic compounds, including flavonols (quercetin, catechins), tannins, ellagitannins and phenolic acids, but anthocyanins make by far the largest contribution to its phytochemical mix (Upton, 2001; Seeram, 2008). These naturally occurring phenolic compounds are redox-active antioxidants as well as iron chelators (Benzie, 2003; Zafra-Stone *et al.*, 2007) and are found in red, blue and purple-coloured flowers, fruits and vegetables. The usual daily dietary intake of anthocyanins is of approximately 200 mg (Zafra-Stone *et al.*, 2007). Bilberry has higher anthocyanin content compared to other types of berries, such as strawberry, cranberry, elderberry and raspberry (Kowalczyk *et al.*, 2003; Bagchi *et al.*, 2004; Yildirim, 2006; Cravotto *et al.*, 2010; Cocetta, 2012). The total anthocyanin content of bilberry is generally in the range of 300-700 mg per 100 g fresh fruit, although this range varies with cultivar, growing conditions and degree of ripeness of the berry (Upton, 2001; Burdulis *et al.*, 2009). Along with anthocyanins, 100 g of fresh bilberry contain small quantities of vitamin C (3 mg), quercetin (3 mg), and catechin (20 mg) (Upton, 2001; Erlund *et al.*, 2003).

Together with the growing popularity of wild berry consumption, cultivation area of berries broadens in consequence of breeding programs (Koca and Karadeniz, 2009). Pomological features of berries are highly influenced by the species and variety within species and the ecological conditions of the plants (Mikulic-Petkovsek *et al.*, 2014; Veberic *et al.*, 2015). Despite a great range of researches conducted as comprehensive studies on fruit properties of blueberries (Smolarz, 2006; Beccaro *et al.*, 2006; Celik, 2009; Giovanelli and Buratti, 2009; Zorenc *et al.*, 2016), the knowledge available on bilberries is scarce.

To our best knowledge, the hereby is the first study conducted on fruit characteristics of wild bilberry population native to Northeastern Anatolia. Hence, the objective of the investigation was to obtain data about fruit characteristics of wild bilberry population grown naturally in Coruh valley located in Northeastern Anatolia of Turkey.

Materials and Methods

Plant material

In the current study, ripe berries of wild *V. myrtillus*, grow naturally in Coruh valley were sampled. The common blueberry cultivar 'Bluecrop' was also included in the study. Berries were sampled at dark-blue color in mid-August, during 2014 and 2015, from 8 pre-selected bilberry accessions ('Coruh-1' til 'Coruh-8') that have better horticultural characteristics (high yield, free pest and diseases, having bigger fruits).

Determination of physicochemical fruit characteristics

Fruit weight, external fruit colour (L, a, b values), titrable acidity and soluble solid content (SSC) were determined on a total 50 fruits per accession in respect to five replications, with ten berries in each replication. Fruit weight values of each fruit were measured with an electronic balance of 0.01 g sensitivity. Skin colour of fruits was measured with a Minolta Chroma Meter CR-400 (Minolta-Konica, Japan). Chroma meter was calibrated to a standard white reflective plate and used Commission Internationale de l'Eclairage (CIE) illuminant C. L* (lightness), a* (green to red) and b* (blue to yellow) values were measured. Colour readings were taken four times at the equatorial region of each fruit and averaged to give a mean value for each fruit (Barnalte *et al.*, 2003). Fruit firmness was measured with a non-destrictive computerized device. The firmness for 50 randomly selected berries from each replicate was expressed as a gram-force causing fruit surface to bend 1 mm. The fruits were peeled manually and cut into small pieces and juiced. The soluble solid content (SSC) was measured in the filtered juice using a digital refractometer. The juice was also analysed for pH and titratable acidity, which determined following the guidelines of the official AOAC method (AOAC, 1995).

Determination of bioactive contents

Sample preparation and extraction procedure

For the total phenolic, total anthocyanins and total antioxidant capacity analyses, harvested berry samples were frozen and stored at -20 °C until analysis. After thawing to room temperature, triplicate of 100 g lots of bilberry and blueberry berries from each accessions/cultivar were homogenized in a blender and they were screened for their total phenolic, total anthocyanin and antioxidant capacity following a single extraction procedure (Singleton and Rossi, 1965). For this procedure, 3 g aliquots of each homogenate were transferred to polypropylene tubes and extracted with 20 mL of extraction buffer containing acetone, deionized water, and acetic acid (70:29.5:0.5 v/v), for one hour.

Total phenolic content

Total phenolic contents were measured according to Singleton and Rossi (1965). To determine the levels of total phenolics, 1 mL of each extract was combined with Folin-Ciocalteu's phenol reagent and water 1:1:20 (v/v) and incubated for eight minutes, followed by the addition of 10 mL of 7% (w/v) sodium carbonate. After two hours, the absorbance of each was measured at 750 nm. The values of total phenolic were estimated by comparing the absorbance of each with those of a standard response curve generated with gallic acid. Results were expressed as μg gallic acid equivalents on a fresh weight (FW) basis (mg GAE per 100 g fresh weight).

Total anthocyanin content

Total anthocyanin content were estimated by a pH differential method (Giusti and Wrolstad, 2005) using a UV-vis spectrophotometer. Absorbance was measured at 533 and 700 nm in buffers at pH 1.0 and 4.5 using $A = (A_{535}–A_{700})_{pH1.0}$ $(A_{535}–A_{700})_{pH4.5}$ with a molar extinction coefficient of 29,600. Results were expressed as mg of cyanidin-3-glucoside equivalent in 100 g fresh weight basis.

Determination of fruit antioxidant capacity

Antioxidant capacity was determined with FRAP (Ferric reducing antioxidant power) assay. In FRAP assay, 2.95 mL aliquot of a FRAP reagent, a mixture of 0.1 mol/L acetate buffer, 10 mmol/L TPTZ and 20 mmol/L ferric chloride (10:1:1 v/v/v), were combined with 50 µL of acetone fruit extract. To determine the antioxidant capacity of the samples, the absorbance values were compared with those obtained from the standard curves of trolox (10-100 µmol/L). The antioxidant capacity values were expressed as trolox equivalent µmol per g fresh weight basis (Benzie and Strain, 1996).

Vitamin C

Ascorbic acid (Vitamin C) of samples was quantified with the reflectometer set of Merck Co (Merck RQflex) and expressed mg per 100 g fresh fruit.

Statistical analysis

The experiment was a completely randomized design with five replications. Data were subjected to analysis of variance and means were separated by Duncan multiple range test at $P < 0.05$ significant level. There were no differences between years thus the data of both years were pooled.

Results and Discussion

Physicochemical properties

Some important physicochemical characteristics regarding wild bilberry and blueberry cv. 'Bluecrop' sampled from Coruh valley are shown in Table 1. One-way Anova for all parameters, except fruit weight and b color values, shows that significant differences between samples ($p < 0.005$); data put in evidence that wild bilberry fruits clearly differentiate from the cultivated blueberry cv. 'Bluecrop' in the main physicochemical properties such as fruit weight, fruit color, fruit firmness, SSC, pH and acidity.

The genotypes exhibited fruit weights from 0.20 to 0.29 g, respectively. The blueberry cultivar cv. 'Bluecrop' had 0.83 g average fruit weight indicating that cultivated blueberry fruits have much larger berries than the wild grown bilberry (Table 1). Previously fruit weight reported were between 0.28-0.29 g for bilberry and 0.80-1.60 g for highbush blueberry in Italy (Giovanelli and Buratti, 2009). In Western part of Turkey fruit weight were found to be between 0.20-0.27 g among 6 wild grown *V. myrtillus* samples (Turkben *et al.*, 2008). The current results are in agreement with the above literature data on fruit weight.

L, a and b external fruit colour values of *V. myrtillus* genotypes and blueberry cv. 'Bluecrop' are given in Table 1. The L, a and b values were found between 11.8-18.3; 0.48-0.69 and 0.89-1.45 among wild bilberries and were 32.4, 0.68 and 4.71 in blueberry cv. 'Bluecrop'. Turkben *et al.* (2008) observed fruit L, a and b colour values between 11.26-16.69; 1.55-4.17 and 0.34-0.93 among 6 wild grown *V. myrtillus* samples naturally grown in Western Turkey. The results clearly indicate that wild bilberries are darker fruits than the cultivated ones, as demonstrated by the lower L values. The current data are in conformity with findings of other researches on bilberry and blueberry (Prior *et al.*, 1998; Moyer *et al.*, 2002; Giovanelli and Buratti, 2009).

Fruit firmness was found between 79 and 88 G/mm among bilberry accessions indicating statistically no differences among them. However, blueberry cv. 'Bluecrop' had higher fruit firmness (277 G/mm) than all wild bilberry samples in the study. Ochmian *et al.* (2009) reported fruit firmness as 86 G/mm in wild bilberries grown in Poland, indicating similarity with the hereby study. Fruit firmness is an important parameter for mechanical resistance, as well as for transport and storage suitability. Low firmness of bilberry fruits indicates softness of these perishable fruits.

SSC, pH and titratable acidity values of the 8 wild grown bilberries and blueberry cv. 'Bluecrop' are shown in Table 1. The highest SSC was detected in the blueberry cv. 'Bluecrop' as 13.3%. Among the bilberry accessions, SSC was found between 10.9% ('Coruh-3') and 11.8% ('Coruh-6') (12.5%). Turkben *et al.* (2008) and Giovanelli and Buratti (2009) reported SSC between 9.0-11.0% and 10.8-11.1% among wild grown *V. myrtillus* samples in Italy and Turkey, respectively. Oancea *et al.* (2013) reported SSC between 9.2-13.7% among wild bilberries in Romania indicating similarities with the current results. Ochmian *et al.* (2009) also reported SSC as 13.0% in wild bilberries grown in Poland. Different growth and environment conditions such as day length, light intensity, temperature, greatly influenced the SSC of bilberries as shown by Martinussen *et al.* (2012).

pH and titratable acidity of the 8 wild grown bilberry were found between 2.85-3.07 and 0.88-1.05%, respectively (Table 1). The blueberry cv. 'Bluecrop' had pH 2.85 and titratable acidity 1.31%. Turkben *et al.* (2008) reported pH and titratable acidity between 2.77-2.95 and 0.90-1.23 among wild bilberries from western Turkey. Giovanelli and Buratti (2009) reported pH and titratable acidity in the range of 3.13-3.22 and 1.00-1.18% which are in accordance to the results for bilberry fruits estimated in this study. Ochmian *et al.* (2009) also reported titratable acidity as 1.44% in wild bilberries grown in Poland, indicating higher values than the samples studied hereby.

Bioactive contents

As indicated in Table 2, statistically significant ($p < 0.05$) variability for anthocyanins, total phenolics and antioxidant activity was observed among the samples (Table 2).

Overall, bilberry samples had higher total phenolic, total anthocyanin and antioxidant capacity than blueberry cv. 'Bluecrop'. However cv. 'Bluecrop' had higher vitamin C content than all bilberry samples (Table 2) .Total phenolic content of bilberry genotypes ranged from 492 ('Coruh-4') to 563 ('Coruh-7') mg GAE per 100 g fresh fruit. The blueberry cultivar 'Bluecrop' had total phenolic as 327 mg GAE per 100 g fresh fruit, indicating lower values than all wild bilberry genotypes. The differences in total phenolic amounts expressed as GAE were deemed to be caused by genetic variation, as all accessions were grown under the same ecological conditions. The average amount of total phenolic content in the fruit of *V. myrtillus* has been previously reported in Italy as 577-614 mg GAE per 100 g (Giovanelli and Buratti, 2009), 640 mg GAE per 100 g fresh bilberry fruit in Poland (Ochmian *et al.*, 2009), 429-671 mg GAE per 100 g blberry fruit in Montenegro (Jovancevic *et al.*, 2011), respectively. Oencea *et al.* (2013) also reported total phenol as 355 mg GAE per 100 g in bilberry and 110 mg GAE per 100 g fruit in blueberry cv. 'Bluecrop'. Prior *et*

Table 1. Physicochemical characteristics of 8 bilberry accessions and blueberry cv. 'Bluecrop' (means for time interval 2014-2015)

Accessions	Fruit weight (g)	Fruit external color			Fruit firmness G/mm	SSC (%)	pH	T. acidity (%)
		L	a	b				
'Coruh-1'	0.22b	12.7bc	0.51^{NS}	1.08b	81b	11.3bc	2.96b	1.00bc
'Coruh-2'	0.27b	16.2bc	0.60	0.89b	83b	11.5bc	2.85c	0.93bc
'Coruh-3'	0.29b	15.3bc	0.48	1.37b	85b	10.9c	2.90bc	1.04bc
'Coruh-4'	0.25b	17.1bc	0.65	1.45b	81b	11.7bc	3.02ab	1.02bc
'Coruh-5'	0.22b	11.8c	0.50	1.28b	79b	11.5bc	3.07a	0.90c
'Coruh-6'	0.25b	14.9bc	0.69	1.17b	83b	11.8b	3.00ab	0.88c
'Coruh-7'	0.20b	18.1bc	0.50	1.36b	85b	11.0bc	2.87bc	1.05b
'Coruh-8'	0.26b	18.3b	0.57	1.41b	88b	11.1bc	3.04ab	0.95bc
'Bluecrop'	0.83a	32.4a	0.68	4.71a	277a	13.3a	2.85c	1.31a

Different letters indicate the statistical difference within same column among accessions at 5% level

Table 2. Bioactive characteristics of bilberry accessions and blueberry cv. 'Bluecrop' (means for time interval 2014-2015)

Accessions	Total phenolics	Total anthocyanins	Vitamin C	Antioxidant activity (FRAP)
'Coruh-1'	522bc	315bc	8b	44.2cd
'Coruh-2'	507c	302cd	4b	52.4ab
'Coruh-3'	530bcd	307c	5b	50.0ab
'Coruh-4'	492d	301cd	7b	40.3cd
'Coruh-5'	534b	318bc	6b	43.5cd
'Coruh-6'	550ab	320bc	7b	47.8b
'Coruh-7'	563a	342a	6b	57.6a
'Coruh-8'	498cd	324b	7b	54.8ab
'Bluecrop'	327e	142e	39a	26.1d

Different letters indicate the statistical difference within same column among accessions at 5% level

al. (1998) reported concentrations of total phenolics ranging from 233 to 273 mg GAE per 100 g in blueberry (*V. corymbosum*) cultivars and 525 mg GAE per 100 g in bilberry (*V. myrtillus*) accessions.

The amounts of total anthocyanin content in fruits of *V. myrtillus* genotypes expressed as cyanidin 3-glucoside ranged from 307 to 342 mg per 100 g. Previous studies on bilberry have reported a variation of 330-344 mg per 100 g of total anthocyanin content (Giovanelli and Buratti, 2009) and 300-698 mg per 100 g (Mazza and Miniati, 1993), which is in in accordance with the current results. Oencea *et al.* (2013) reported average total anthocyanin content as 273 mg expressed as cyanidin 3-glucoside per 100 g in bilberry and 99 mg per 100 g fruit in blueberry cv. 'Bluecrop'. Jovancevic *et al.* (2011) reported average 360 mg total anthocyanin expressed as cyanidin 3-glucoside in bilberry samples in Montenegro. Beccaro *et al.* (2006) found 327 mg anthocyanin in bilberry fruits. Anthocyanins comprise the major part of the phenolic profiles in bilberry fruits (Oancea *et al.*, 2013). Anthocyanins are distributed mainly in the skin of cultivated blueberries (*Vaccinium corymbosum* L.) and both in skin and flesh of wild bilberries (*V. myrtillus* L.) also known as European blueberry. The berries of *V. myrtillus* had higher anthocyanin content than highbush blueberry, because of their smaller size (and greater skin/flesh ratio) and due to the red colour of the bilberry flesh. Anthocyanins have potential health benefits independent or additional to their antioxidant effects (Morazzoni and Bombardelli, 1996).

All bilberry samples had low vitamin C content which was found to be between 4 and 8 mg per 100 g fresh samples. However, blueberry cv. 'Bluecrop' had exceptionally higher vitamin C content (39.10 mg per 100 g) compared to wild bilberries. Due to low Vitamin C content of bilberry fruits, there were few studies in literature on this phenomena. Ochmian *et al.* (2009) reported average 7 mg Vitamin C in 100 g bilberry fruits and 34 mg in cultivated blueberry cv. 'Duke' in 100 g fruits in Poland. Noormets *et al.* (2006) reported average 6 mg Vitamin C in bilberry fruits in Estonia.

Within the present study, the total antioxidant capacity of bilberry samples was found between 40.3-57.6 expressed as μmol trolox equivalent per g in FRAP method. The blueberry cultivar 'Bluecrop' had 26.1 μmol trolox equivalent per g, indicating a lower value than all bilberry accessions studied (Table 2). Giovanelli and Buratti (2009) found the iron-ion-reducing antioxidant power values in the fruits of *V. myrtillus* genotypes as 53-57 μmol trolox equivalent per g. They also reported these values as 24 μmol trolox equivalent per g in blueberry cv. 'Bluecrop'. Beccaro *et al.* (2006) reported 3 times higher antioxidant activity in bilberry fruits than blueberry cultivars determined by FRAP method. To the best of our knowledge, the antioxidant activity of *V. myrtillis* has not been previously evaluated by the FRAP assay in Turkey and in literature there were few results on this phenomena.

Conclusions

The current results indicated that the wild bilberry and cultivated blueberry fruits had different pomological characteristics and bioactive contents of polyphenols, vitamin C, anthocyanins and antioxidant capacity.

Moreover, bilberry fruits had higher human health compounds such as polyphenols and anthocyanins compared to blueberry. The results indicated that the fruits of wild-grown bilberry possess strong antioxidant activities. The cultivation of bilberry is not difficult, therefore the fruits of this plant could be a prospective source of natural bioactive molecules that could replace synthetic antioxidants and serve as a potential source of antibacterial agents in the food industry.

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