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Original Article

Antioxidant Activity, Sugar Content and Phenolic Profiling of Blueberries Cultivars: A Comprehensive Comparison

Onur Tolga OKAN[1]*, Ilhan DENİZ[2], Nurettin YAYLI[3], Ihsan Güngör ŞAT[4], Mehmet ÖZ[5], Gönül HATİPOĞLU SERDAR[3]

[1]*Karadeniz Technical University, Technology Transfer Office, 61080, Trabzon, Turkey; onurtolgaokan@ktu.edu.tr (*corresponding author)*
[2]*Karadeniz Technical University, Faculty of Forestry, Department of Forest Industrial Engineering, 61080, Trabzon, Turkey; ideniz@ktu.edu.tr*
[3]*Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosis, 61080, Trabzon, Turkey; yayli@ktu.edu.tr; gonulserdar@ktu.edu.tr*
[4]*Ataturk University, Faculty of Agriculture, Department of Food Engineering, 25240, Erzurum, Turkey; igsat@atauni.edu.tr*
[5]*Gümüşhane University, Gümüşhane Vocational School, Department of Forestry, 29100, Gümüşhane, Turkey; mehmetoz@gumushane.edu.tr*

Abstract

Commercial blueberry production has been a viable industry throughout the world for 95 years; because of blueberry is a good source of antioxidant. Blueberries are especially rich in anthocyanin, a flavonoid with potent antioxidant capacity. The aim of this study was to compare the phenolic quantities, antioxidant activities, anthocyanin, sugar and phenolic compounds of blueberries produced in Turkey with those of similar blueberry varieties produced around the world. As a result of the conducted analysis, the total phenolic content (TPC) amount found in the berries was 77.26-215.12 mg GAE/100 g, the total flavonoid content (TFC) was 30.44-91.69 mg QE/100 g and the total anthocyanin content (TAC) was 43.03-295.06 mg c3-GE/100 g. Examining the antioxidant activities of the berries, DPPH between 1.10-5.65 mg/ml, FRAP between 454.93-36832.96 µmol troloks/100 g, β-Carotene between 40.66-86.48%. It was determined that the natural berries contained much more phenolic compounds and higher antioxidant activity than that of the cultivars The result of HPLC analysis, chlorogenic acid is determined to be the dominant compound in all berries. Furthermore, fructose and glucose are found in all fruits in different quantities while sucrose is found in certain varieties of berries as well. At the end of the performed study the data indicate that wild and cultivars of blueberries are rich sources of antioxidants for local as well international industries importing this fruit for food processing and enormous products.

Keywords: antioxidant; blueberry; non-wood forest; phenolic content; sugar analysis

Introduction

Non-wood forest products (NWFPs, berries, wild herbs, mushroom) are bio products which are commonly consumed worldwide. NWFP are typically used in functional and premium class foodstuffs and nutraceuticals (Peltola, 2013). The recent global interest in the consumption of foods with high levels of functional properties and nutraceuticals compounds is gaining momentum (Zimmer *et al*., 2014). Among these types of foods, berries are one of the most important functional and nutraceutical foods in our diets (Kähkönen *et al*., 2001). Phenolic compounds are widely distributed in such plants where they act as attractants for certain insects, as free radical scavengers, and in defence against ultraviolet radiation, pathogens and predators (Zimmer *et al*., 2014; Solovchenko and Schmitz-Eiberger, 2003). In this context, blueberries (*Vaccinium* spp.) are known for being rich in bioactive compounds, including flavonoids, phenolic acids, tannins and anthocyanin's (Yang *et al*., 2014). The antioxidant capacity is always significantly correlated with the contents of these compounds (Kalt *et al*., 2000). The antioxidant activity of blueberries is relatively the most prominent amongst different bioactive properties (Häkkinen and Törrönen, 2000). Thus, blueberries are popular in grocery stores and are sold fresh as well as in processed forms such as in beverages, yogurt, jelly and jam (Seeram *et al*., 2006).

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Several studies were conducted to assess the antioxidant and phenolic capacity in wild and cultivar blueberries with the aim of obtaining comparative data and identifying the effect of regional variations (Taruscio *et al*., 2004; Brambilla *et al*., 2008; Yang *et al*., 2014). In this regard, the profile of flavonoid and phenolic compounds in nine *Vaccinium* species which included domestic blueberry cultivars were examined (Taruscio *et al*., 2004). It was found that there were inter and intra species differences between berry groups in total phenolics (TPH), anthocyanins (ACY) and antioxidant capacity. Catechin, epicatechin, myricetin and quercetin from flavan-3-ol and flavonol and caffeic, chlorogenic, *p*-coumaric, ferrulic and *p*-hydroxybenzoic from phenolic acid in *V. corymbosum* and *V. angustifolium* × *V. corymbosum* cultivar species were also detected. *V. corymbosum* and *V. angustifolium* × *V. corymbosum* cultivar contained a high amount of quercetin, exhibiting 86.4-102.5 μg/g and a high amount of chlorogenic acid, exhibiting 1261-1414 μg/g (Taruscio *et al*., 2004). Another study (Yang *et al*., 2014) revealed the detection of gallic acid, caffeic acid, vanilic acid, syringic acid, *p*-coumaric acid and ferrulic acid in *Vaccinium*, while quercetin and kaempferol were not detected, however they found lower total phenolic content and a higher antioxidant capacity is shown by *Vaccinium* fruits. Another group studied (Brambilla *et al*., 2008) the phenolic profile and antioxidant capacity of juice made from a *Vaccinium corymbosum* cultivar. They reported that the main compound of juices from *Vaccinium* cultivars is chlorogenic acid. Also, the total content and relative distribution in anthocyanin's, chlorogenic acid, and quercetin of each juice was dependent upon the cultivar, and the total content was highly correlated to the antioxidant capacity. Climatic factors are effective on blueberry bioactive compounds. Annual and geographical factors appear to influence antioxidant value, although many years of study are needed to distinguish these effects from other biotic and abiotic factors that influence fruit phenolic content (Kalt *et al*., 2001).

Although blueberries are now grown commercially in the Southern Hemisphere in Australia, New Zealand and South American nations, until the 1930s (when introduced in Europe) blueberries were limitedly native to North America and commercially cultivated as highbush blueberries (Gao and Draper, 2010). Naturally acidic soils, which blueberries grow best (Ochmian *et al*., 2015), are found in the Black Sea region of Turkey. This region is also where the native blueberry (low bush) species have grown for hundreds of years. However, in the year 2000, the highbush blueberry cultivation started in Turkey were introduced from some western countries those originally gotten from USA. Apart from Turkey, wherever high bush blueberry cultivation is done, the grown plants have been studied for their bio significance and chemical composition (Celik, 2009).

Interestingly so far, no studies have been conducted on the blueberry cultivar samples in Turkey which are currently consumed not only by the Turkish population but are also exported to other countries as fresh berries and in different forms of processed foods (Ercisli and Celik, 2009).

In light of this, the purpose of our comprehensive study was to examine the phenolic composition, total anthocyanin, total phenols, and antioxidant activity of wild and cultivated blueberries grown in Turkey. It is important to understand the relationship between phenolic and agronomical parameters or future selection of blueberry genotypes having improved nutritional quality when consumed as fresh or as processed blueberry products.

Materials and Methods

Chemicals

Folin-Ciocalteu's phenol reagent, 2,4,6-Tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Standard phenolic compounds were obtained from Sigma-Aldrich (Steinheim, Germany). The rest of the reagents used in the study were obtained from Merck, Darmstadt, Germany through its local authorized distributors.

Plant material

A total of 28 samples of blueberries were obtained from the Black Sea region situated in north eastern Turkey, during the peak growing season in 2012, 2013 and 2014. Berries were harvested from Giresun (Bulancak), Trabzon (Hayrat and Kaşüstü) and Rize provinces. The samples were immediately frozen and stored at -45 °C. (Table 1).

Fruit extraction for antioxidant activity and phenolic analysis

Approximately 16 g of each blueberry sample was added to an equal volume (40 mL) of 99% methanol and homogenized in a blender for 3 minutes. The mixture was continuously stirred with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h. Particles were removed with filter paper. The final volume of the solution was adjusted with methanol. The methanolic extract was divided into two parts, the first being used for antioxidant tests and the second for phenolic analysis with HPLC. The liquid-liquid extraction procedure was applied to the second part for phenolic extraction. For each berry sample extract (prepared in methanol), 100 mg of the sample was dipped in pH 2.0 ± 0.1 water and shaken vigorously followed by extraction with diethyl ether and ethyl acetate (3×5 mL each). Organic phases were combined evaporated and made up in methanol (2.0 mL) for HPLC after passing through filter 45μ size.

Fruit extraction for sugars

Fruit extractions were carried out according to the method described by (Kafkas *et al*., 2006) with some modifications. Fruit samples were dried in an oven at 45 °C for one week. Dried samples were powdered by a crusher and approximately 1 g of each sample was weighed. Powdered fruit samples were transferred to a screw cap Eppendorf tube with 20 mL of aqueous ethanol (80% v/v). A reaction mixture was placed in a shaker and shaken at room temperature for 24 h/200 rpm. Particles were removed with filter paper and liquid part evaporated to dryness with evaporator. The residue was dissolved with 2 ml of distilled water and filtered before HPLC analysis.

Table 1. List of blueberry sampling place, collected year and cultivar

Year	Place	Type	Cultivar
2014	Giresun-Bulancak	NHB	Sunshine
2014	Giresun-Bulancak	NHB	Northland
2014	Giresun-Bulancak	SHB	Ozarkblue
2014	Giresun-Bulancak	SHB	Misty
2014	Giresun-Bulancak	NHB	Bluegold
2014	Giresun-Bulancak	NHB	Sunrise
2014	Giresun-Bulancak	SHB	Jubile
2012	Rize-Handüzü	N	*Vaccinium arctostaplylos*
2012	Rize-Handüzü	N	*Vaccinium myrtillus*
2014	Trabzon-Hayrat	NHB	Brigitta
2014	Trabzon-Kaşüstü	NHB	Duke
2014	Giresun-Bulancak	SHB	Oneil
2014	Giresun-Bulancak	NHB	Darrow
2014	Trabzon-Kaşüstü	NHB	Torro
2014	Trabzon-Kaşüstü	NHB	Herbert
2014	Giresun-Bulancak	NHB	Brigitta
2014	Giresun-Bulancak	NHB	Chandler
2014	Trabzon-Kaşüstü	NHB	Blueray
2014	Trabzon-Hayrat	NHB	Jersey
2014	Trabzon-Hayrat	NHB	Bluecrop
2012	Rize	NHB	Earlyblue
2014	Trabzon-Hayrat	NHB	Bluegold
2014	Trabzon-Kaşüstü	NHB	Putte
2014	Trabzon-Kaşüstü	NHB	Berkeley
2014	Trabzon-Hayrat	NHB	Torro
2014	Trabzon-Kaşüstü	NHB	Patriot
2014	Trabzon-Kaşüstü	NHB	Bluejay
2014	Giresun-Bulancak	NHB	Bluejay
2014	Giresun-Bulancak	NHB	Bluecrop
2014	Trabzon-Kaşüstü	NHB	Spartan
2014	Trabzon-Kaşüstü	NHB	Puru Sampling
2013	Rize-Handüzü	N	*Vaccinium arctostaplylos*
2013	Rize-Handüzü	N	*Vaccinium myrtillus*
2014	Trabzon-Kaşüstü	NHB	Legassi
2014	Trabzon-Kaşüstü	HH	Northcountry

Determination of total phenolic contents (TPC)

TPC's of methanolic extracts were determined with a method previously used and reported by Folin-Ciocalteu (Singleton *et al*., 1999). Briefly, 750 µL of Folin-Ciocalteu's/water mixture (1:14) was added to a 50 µL sample and after 3 min 200 µL of 20% Na_2CO_3 was added. Then the reaction mixture was incubated in the dark for 30 min. The absorbance was measured on an ultraviolet-visible (UV–Vis) spectrophotometer (Unicam UV2-100) at 760 nm and methanol was used as blank. Gallic acid was used as a standard and total phenol contents in extracts were calculated as mg gallic acid equivalent total phenolic in mg Gallic Acid Equivalent/100 g (mg GAE /100 g) dry weight of plant.

Determination of total flavonoid content (TFC)

The total amount of flavonoid was measured using the spectrophotometric method at 430 nm as reported previously (Lamaison and Carnart, 1991). Stock solutions of each extract were prepared in methanol (4 mg/ml). A 1.5 ml of a 2% methanol solution of $AlCl_3.6H_2O$ was added to 0.5 ml of the sample, then the sealed bottles were kept in the dark for 10 min. The absorbance was read at 430 nm, methanol $AlCl_3$ used as blank, each measure in triplicated. A series of dilutions of quercetin in methanol was prepared and assayed; flavonoid amounts in the extract were expressed as mg quercetin equivalent flavonoid in mg quercetin Equivalent/100 (mg QAE /100 g) dry matter.

Determination of anthocyanin content (TAC)

Stock solutions of each extract were prepared in methanol (6 mg/ml). Two 40 µL portions of the methanol stock solution were put into the test tubes and nine hundred and sixty microliters of pH 1.0 (25 ml of 1.49% KCL+ 67 ml of 1.7% HCl, pH corrected with HCl) or pH 4.5 (1.64% AcONa, pH corrected with AcOH) buffer solutions were added. The absorbance was read at 700 and 510 nm against blank for both pH values. Each experiment

was carried out in triplicates and the total anthocyanin content was calculated from the following equation (Giusti and Wrolstad, 2001).

$\Delta A = [(A_{510nm} - A_{700nm})]_{pH=1.0} - [(A_{510nm} - A_{700nm})]_{pH=4.5}$

TACY = (ΔA x MWx DF x 1000)/ε) x 0.1

TACY = total anthocyanins expressed as mg cyanidin 3-glucoside/100g sample

MW = molecular weight of cyanidin 3-glucoside (449.2 g/L).

DF = dilution factor to express the samples on a per gram of plant

1000 is the conversion factor for grams to mg.

ε = molar absorbance coefficient of cyanidin 3-glucoside (26,900 L $M^{-1}cm^{-1}$).

0.1 is the conversion factor for per 1000 grams to 100 gram basis.

Antioxidant activity assays

DPPH antioxidant activity has become a general test method, because of rapid, simple and independent of sample polarity for measurement of free radical scavenging ability of plant extracts. The DPPH free radical scavenging activity of the extract was determined by a previously reported method (Kartal *et al*., 2007). The scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to determine the radical scavenging activity of the methanolic blueberry samples. The colorimetric test was assayed using the Molyneux method. DPPH radical has a purple colour which decays in the presence of antioxidant agents, thus the change of the absorbance is monitored at 517 nm. Tests were conducted in triplicates and butylated hydroxyl toluene (BHT) was used as a positive control.

β-carotene-linoleic acid inhibition activity

The β-carotene-linoleic acid inhibition activity of the extract was determined using a previously reported method (Huang *et al*., 2005). Briefly, 0.5 mg β-carotene was dissolved in 1 mL chloroform then 25 µL linoleic acid and 200 mg Tween 40 were added to this and mixed vigorously. The chloroform was then evaporated under reduced pressure on a rotary evaporator and 100 ml of oxygenated distilled water was subsequently added to the residue and mixed gently to form a clear yellowish emulsion. The 350 µL of extract (2 mg/mL in ethanol) was placed into a test tube and 2.5 mL of β-carotene-linoleic acid mixture was added to this and mixed thoroughly. The mixture was incubated at room temperature for 24 hours and then the absorbance was measured at 490 nm on an ultraviolet-visible (UV–vis) spectrometer. BHT was used as a positive control and for a negative control (blank) the same volume of ethanol was used instead of the extract. Reading of blank was taken before and after the 24 hour incubation process and the absorbance value was subtracted from all samples. Relative antioxidant activity (RAA %) of the extract was calculated using the following equation:

$$RAA\% = \frac{A\ sample}{A\ BHT} \times 100$$

FRAP (Reducing Ability) Assay

The measure of extract to reduce the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex is also considered as an antioxidant activity. The assay protocol was carried out according to the methods described by Benzie and Strain (2005), with some modifications. The test involves the reduction of ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to a blue colored Fe (II) TPTZ by antioxidant constituents of extracts. Working ferric reducing/antioxidant power (FRAP) reagent was prepared by mixing (10:1:1) of 300 mM acetate buffer (pH 3.6) with of 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution. 3 mL of the freshly prepared FRAP reagent and 100 µL of the samples were mixed and incubated for 4 min at 37 °C and the absorbance was noted at 595 nm against reagent blank containing distilled water. Trolox was used a positive control to construct a reference curve (62.5-1000 mg/L), FRAP values were expressed as mg Trolox Equivalent/100g (mg TE/100 g).

Analysis of phenolic compounds by HPLC

Seventeen standards of phenolic compounds were analysed using HPLC (Fig. 1). HPLC-DAD analysis of biomass methanol extract was performed according to the method described by Hatipoğlu *et al*. (2013), with some changes in the gradient flow of the mobile phase. The HPLC-DAD system (Agilent Technology, 1260 infinity) consisted of quaternary pumps (1260 QUAT pump VL) and an auto injector (model 1260 ALS) connected to a DAD (diode array detector) (1260 DAD VL). An AC-18 reverse phase column (250 mm × 4.6 mm id, 5 µm particle sizes, HICHROM, UK) was used for the analysis which was fixed in the column oven (1260 TCC). The mobile phase was a mixture of solvent A (2% AcOH in water) and solvent B (70:30, acetonitrile/water) which was sonicated before stirring and continuously degassed by the built-in HPLC system. The injection volume was 20 µL and the column was kept at 30 °C. The calibration curves for quantification were obtained by running reference standards in the range of 1.5 to 25 ppm and the regression for all phenolics was found ≥ 0.999. The flow rate was kept constant at 1 mL min^{-1} using gradient programming; starting the flow of mobile phase as B (5%) to three minutes, gradually increasing (up-to 15, 20, 25, 40 and 80% at 8, 10, 18, 25 and 35 minutes respectively) and decreasing to 5 % at 40 minutes and left for 10 minutes to equilibrate in the column. The eluent was continuously monitored through PDA by measuring at three different wave lengths i.e. 280, 315 and 350 nm.

Analysis of sugar compounds by HPLC

Ten standards of sugar compounds were analyzed using HPLC-RID (Fig. 2). The liquid chromatographic apparatus shows the same features with phenolic analysed using HPLC. Refractive index detector is used for sugar analysis as distinct from phenolic analysis. Separations were performed on a reverse-phase Nucleosil NH_2 analytical column operating at room temperature with a flow rate of 1 mL min^{-1}. The sample injection volume was 20 µL. The calibration curves for quantification were obtained by running reference standards in the range of 1.5 to 25 ppm and the regression for all phenolics was ≥ 0.999. Elution was effected using an isocratic elution of 79% aqueous acetonitrile as a solvent. Compounds were identified by comparing their retention times. 10 min equilibrium time was allowed between injections.

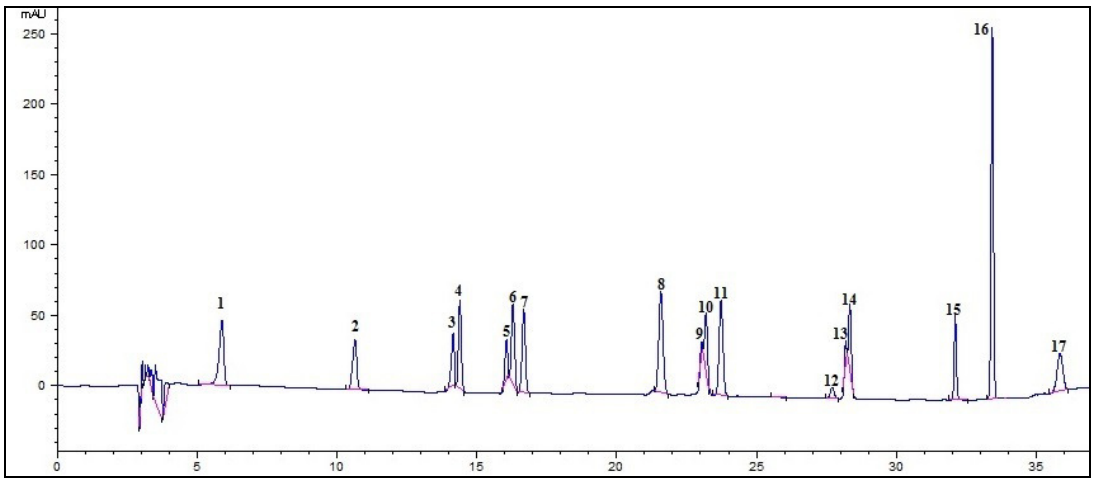

Fig. 1. HPLC-DAD chromatograms of the Phenolic Standards (280 nm) (1) Gallic acid, (2) Protocatechuic acid, (3) Chlorogenic acid, (4) p-OH benzoic acid, (5) Vanilic acid, (6) Kaffeic acid, (7) Syringic acid, (8) Ferulic acid, (9) Ellagic acid, (10) Rutin, (11) *p*-Kumaric acid, (12) Benzoic acid, (13) Rosmarinic acid, (14) o-cumaric acid (15) quercetin, (16)*t*-cinnamic acid, (17) Curcumin

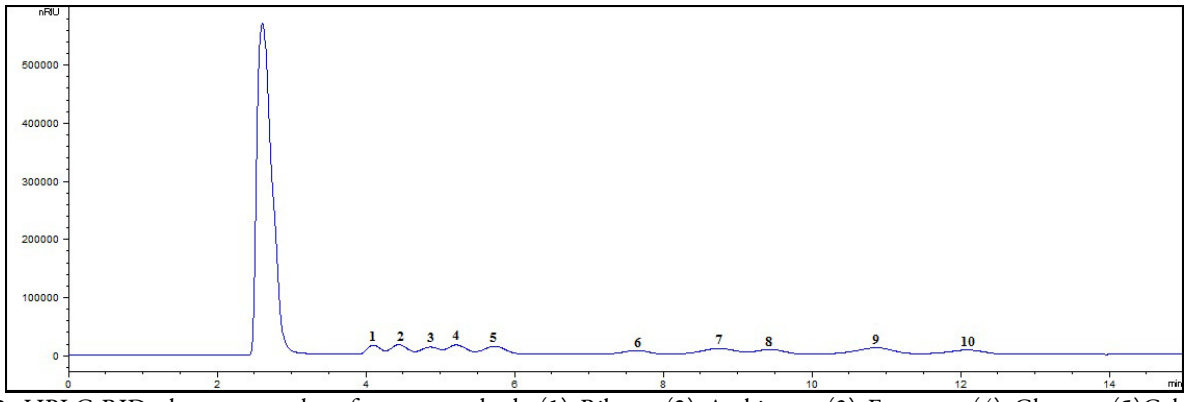

Fig. 2. HPLC-RID chromatography of sugar standards (1) Ribose, (2) Arabinose, (3) Fructose, (4) Glucose, (5)Galactose, (6)Sucrose, (7) Maltose, (8) Trehalose, (9) Melibiose, (10) Melezitose

Statistical analysis

Correlations and 't' tests were performed using SPSS 13.0. The regression and correlation analysis were performed with Kruskal-Wallis and the Pearson correlation analysis as a non-parametric test. The significance was set at $p<0.01$. In the interpretation of the results according to the correlation, $r<0.2$ was assessed as a very weak correlation or no correlation at all, 0.2-0.4 as a weak correlation, 0.4-0.6 as a medium correlation, 0.6-0.8 as a high correlation and 0.8>as a very high correlation.

Results and Discussion

Total phenolic, flavonoids and anthocyanin content

The comparative results regarding the total phenolic content (TPC), total flavonoids content (TFC) and total anthocyanin's content (TAC) in wild and cultivated blueberries are given in Table 2.

TPC is marker of blueberry antioxidant capacity and is generally used as an antioxidant test. The amount of TPC changed from 76.20 mg GAE/100 g to 215.12 mg GAE/100 g also these values were found statistically significant. The total phenolic content in wild species of blueberries was found to be higher than that in cultivar berries. Similar results were reported by Koca and Karadeniz (2009), on *V. arctostaphylos* samples: these authors reported total phenolic concentrations ranging from 308-542 mg/100 g in *V. arctostaphylos,* and a total phenolic content ranging from 77-140 mg/100 g in berries ('Rekord', 'Northland', 'Ivanhoe'). Lee *et al*. (2004), also studied the TPC of wild *Vaccinium* species as compared to cultivated ones. The authors reported that the total phenolic content of wild *Vaccinium* species varied from 489 to 702 mg/100 g, while cultivated *V. membranaceum* total phenolic concentrations ranged from 225 to 423 mg/100 g. Among the cultivated berries, 'Bluejay' (Bulancak) had the highest TPC at 213.82 mg GAE/100 g, while the lowest value was found for the 'Toro' (Hayrat) variety (76.20 mg GAE/100 g). The difference observed in TPC can be attributed to the different locations as well as the fact that synthesis phenolic compounds are affected by various abiotic and biotic factors, including temperature, irradiation, herbivory, and pathogenic infection (Kalt *et al*., 2001). The TPC data obtained are comparable to previous findings which reported values between 251-310 mg GAE/100 g for cultivated blueberries and 577-614 mg GAE/100 g for wild Italian blueberries (Giovanelli and Buratti, 2009). Ehlenfeldt and Prior (2001), reported a higher TPC value

Table 2. Total polyphenols, total flavonoids and total anthocyanin content in blueberries

Sample Name	TPC (mg GAE/100 g)	TFC (mg QE/100 g)	TAC (mg c3-GE/100 g)
Berkeley (Kaşüstü)	140.95±5.92^{mn}	48.64±4.33^{d}	93.50±7.41^{j}
Bluecrop (Bulancak)	83.23±1.09^{ab}	40.36±2.07^{c}	44.97±0.56^{c}
Bluecrop (Rize)	123.51±3.793^{jkl}	59.34±1.36^{f}	55.97±1.48^{d}
Bluegold (Bulancak)	105.57±4.27^{def}	87.55±8.31^{k}	245.89±1.48^{r}
Bluegold (Hayrat)	164.04±11.90^{op}	84.01±1.81^{k}	255.92±8.08^{u}
Bluejay (Bulancak)	213.82±5.34^{v}	57.27±0.51^{ef}	127.79±1.12^{mn}
Bluejay (Kaşüstü)	171.42±3.24^{pr}	58.05±1.18^{f}	156.91±0.56^{p}
Blueray (Kaşüstü)	118.68±2.37^{ghij}	40.02±0.79^{c}	74.41±1.48^{fg}
Brigitta (Bulancak)	134.75±11.29^{m}	41.65±0.79^{c}	86.06±0.56^{hij}
Brigitta (Hayrat)	97.35±6.66^{cd}	37.08±1.44^{bc}	67.29±0.56^{ef}
Chandler (Bulancak)	111.98±1.34^{fghi}	30.44±0.90^{a}	72.47±1.48^{fg}
Darrow (Bulancak)	111.03±1.60^{efg}	41.22±1.61^{c}	111.29±2.96^{k}
Duke (Kaşüstü)	178.55±13.04^{rs}	59.60±0.89^{f}	160.79±4.58^{p}
Early Blue (Rize)	124.24±10.38^{jkl}	55.37±1.16^{ef}	83.47±0.97^{hi}
Herbert (Kaşüstü)	170.57±1.36^{pr}	52.61±0.93^{de}	147.85±2.96^{o}
Jersey (Hayrat)	123.50±6.94^{ijkl}	53.13±0.93^{de}	119.06±1.12^{l}
Jubile (Bulancak)	131.48±2.20^{jklm}	52.61±1.18^{de}	78.29±1.12^{gh}
Legassi (Kaşüstü)	77.26±1.99^{a}	55.37±1.07^{ef}	90.59±4.37^{ij}
Misty (Bulancak)	135.32±3.81^{mn}	48.82±1.27^{d}	22.32±3.36^{a}
Northcountry(Kaşüstü)	92.06±1.30^{bc}	72.45±3.08^{hi}	226.47±6.18^{s}
Northland (Bulancak)	132.97±5.54^{lm}	49.25±1.18^{d}	83.79±6.60^{hi}
Oneil (Bulancak)	101.00±1.35^{cde}	64.69±1.72^{g}	61.14±7.76^{de}
Ozarkblue (Bulancak)	157.24±4.25^{o}	77.72±3.13^{j}	159.18±0.1^{p}
Patriot (Kaşüstü)	118.38±2.84^{ghij}	40.02±0.53^{c}	74.41±2.96^{fg}
Puru (Kaşüstü)	121.83±9.48^{ijk}	37.00±0.59^{bc}	35.25±2.24^{b}
Putte Sampling	170.46±10.04^{pr}	69.52±0.83^{h}	125.53±3.12^{lm}
Spartan (Kaşüstü)	186.51±5.15st	56.32±1.22^{ef}	188.94±3.67^{r}
Sunrise (Bulancak)	120.48±1.49^{hij}	34.49±2.46^{b}	43.03±2.02^{c}
Sunshine (Bulancak)	120.63±1.73^{hij}	55.80±6.21^{ef}	85.09±0.56^{hi}
Toro (Hayrat)	76.20±0.66^{a}	56.58±3.06^{ef}	55.02±3.12^{d}
Toro (Kaşüstü)	108.65±1.92^{efg}	40.45±1.18^{c}	87.35±2.56^{ij}
V. arctostaplylos (2012)	181.35±5.50^{s}	91.69±1.12^{l}	295.06±17.47^{y}
V. arctostaplylos (2013)	193.19±3.09^{tu}	76.34±1.07^{ij}	280.51±5.13^{v}
V. myrtillus (2012)	199.87±2.28^{u}	76.77±2.64^{j}	230.68±2.80^{s}
V. myrtillus (2013)	215.12±1.30^{v}	77.80±3.25^{j}	223.89±2.96^{s}

Different letters (a–z) in the same columns are significantly different at the 5% level ($p < 0.05$).

for 'Berkeley', 'Bluecrop', 'Bluegold', 'Bluejay', 'Brigitta', 'Chandler', 'Duke', 'Earlyblue', 'Herbert', 'Jubile', 'Misty', 'Patriot', 'Puru', 'Spartan', 'Toro' and 'Sunshine' than the the TPC values found for blueberries in our study. The differences in the reported data partially result from varied analytical methods (especially the extraction step) employed by the authors. Similar results, 115.0 and 4.2 mg GAE/100 g for *V. corymbosum* berries in ethanol and water extract, were obtained by Smad *et al*. (2014).

Flavonoids constitute the largest subgroup of the polyphenols, having more than 8,000 compounds in this group. Flavonoids are responsible for antioxidant activity (Pietta *et al*., 2003). In the examination of TFC amounts of the blueberries (Table 2) are determined to have the highest value in *V. arctostaplylos* with 91.69 mg QE/100 g (2011) and the lowest value in the 'Chandler' variety with 30.44 mg QE/100 g. The highest value among the cultivars in the 'Bluegold' variety was found in berries collected from the Bulancak region. Furthermore, there was no statististical difference in regards to their contents for 'Bluegold', 'Bluejay', 'Brigitta' and 'Toro' among berries of the same variety but collected from different regions with the exception of 'Bluecrop'. The total flavonoid contents of natural berries were determined to be relatively higher compared with cultivars. In another study, natural (V. *myrtillus*) and cultivar ('Elliot', 'Bluecrop' and 'Duke') blueberries were examined, the TFC amounts were reported to vary between 84.33-112.5 mg QE/100 g. Moreover, the TFC amount in natural berries was much higher than the TFC amount in cultivar varieties (Bunea *et al*., 2011). It was reported in another study performed by Marinova *et al*. (2005), that the TFC amount of the variety *V. myrtillus* was 190.3 mg CE/100 g (Marinova *et al*., 2005). In the other study, the TPC amounts of 1997, 1998, 1999 of the fruits of the varieties *V. myrtillus, V.uligonosum* and *V. vitis-idea* in the regions Orimattila, Mantyharju,

Nurmes are examined, it is reported that there are some differences determined with regards to the TPC contents of blueberries in natural form grown in different regions of Finland. This difference is particularly significant in regards to the TFC amounts (Kahkönen *et al*., 2001). In addition, a study conducted in northwest Croatia in 2006 and 2007 examined the TFC amounts of 'Duke', 'Elliott', 'Sierra' and 'Bluecrop' berries. The study concluded that the TFC amounts of the cultivar varieties in 2006 and 2007 for 'Duke' were between 268.97 and 216.87 mg RE/100 g, between 376.68 and 255.33 mg RE/100 g for 'Eliott', between 528.15 and 331.34 mg RE/100 g for 'Sierra', and between 368.33 and 291.56 mg RE/100g for 'Bluecrop'. Therefore, this study demonstrates that there are differences between TFC values of cultivar blueberries obtained from the same region during different years. The authors explained that this difference is attributed to changes in the climate in between seasons. Also, researchers argue that particularly high temperatures significantly influence amount of TFC (Uzelac *et al*., 2010). A large amount of literature illustrates that the TFC amounts of the same blueberry can be different. In addition to differences in climatic conditions, there are many variables such as methodological differences at the performed studies or cultivation techniques that may have caused this. (Häkkinen and Törrönen, 2000; Kahkönen *et al*., 2001; Koca and Karadeniz, 2009).

There are 16 different types of anthocyanins responsible for the colouring of blueberries. It is reported as a result of the performed clinic studies that these anthocyanin's significantly increase the night vision (Kalt and Dufor, 1997). The total anthocyanin content (TAC) for blueberries is indicated in Table 2. In the performed study the TAC amount of blueberries varied between 22.32-295.06 mg c3-GE/100 g. It was determined that the TAC amounts in natural blueberries were higher than that in most of the cultivar berries. The highest TAC amount is determined in *V. arctostaplylos* (2012) among the natural varieties and with 255.92 mg c3-GE/100 g in the 'Bluegold' (Hayrat) variety among the cultivar varieties. According to literature, the TAC amounts of blueberries vary between 25-497 mg c3-GE/100 g (Ragvendra *et al*., 2011). The TAC amounts in our study are similar with those in the literature. Previous study about the phenolic compounds of the *V. myrtillus* and 8 different cultivar varieties reported that the TAC amounts of the *V. myrtillus* are higher than those of the other cultivar. They have reported in the same study that the TAC amount for the *V. myrtillus* is 3.70 mg malvidin-3-glucoside/g. The TAC amounts for 'Blomidon', 'Cumberland', 'Fundy', 'Bluecrop', 'Coville' and 'Jersey' were found to be 0.954, 1.53, 2.55, 0.832, 0.998 and 1.17 mg malvidin-3-glucoside/g respectively (Kalt and Dufor, 1997). It was also reported in another study in which eighty seven different blueberry types were examined that the TAC amounts varied between 89-331 mg c3-GE/100 g. The TAC amounts of 'Bluegold', 'Bluejay', 'Legasi', 'Ozarkblue' and 'Spartan' were found to be similar, 'Berkeley', 'Bluecrop', 'Blueray', 'Brigitta', 'Chandler', 'Darrow', 'Duke', 'Earlyblue', 'Herbert', 'Jersey', 'Jubile', 'Legassi', 'Misty', 'Northland', 'Oneil', 'Sunrise', 'Sunshine', 'Patriot', 'Puru' and 'Toro' were found lower than in a previous study (Ehlenfeldt and Prior, 2001). Furthermore, in another study conducted on natural and cultivar varieties in Turkey, the total anthocyanin amounts of blueberry fruits were determined to be between 59-294 mg c3-GE/100 g for *V. arctostaphylos*. In addition, the same study reported the total anthocyanin amounts in 'Jersey', 'Ivanhoe', 'Northland' and 'Record' as 25 mg c3-GE/100 g for 'Jersey' and 'Ivanhoe', 29 mg c3-GE/100 g for 'Northland' and 0,18 mg c3-GE/100 g for 'Record' (Koca and Karadeniz, 2009). Compared with in this study, the TAC amounts of the natural *V. arctostaphylos* berries were found to be quite higher than those of the cultivar forms in the literature, too. In this regard, this study is consistent with the literature. However, there were some instances in which the total anthocyanin amounts for the blueberries were found to be less or much higher than values in the literature. These differences may be due to the fact that anthocyanin synthesis is influenced by environmental biotic and abiotic factors in addition to genotype or different growth conditions (Kalt *et al*., 2000; Koca and Karadeniz, 2009).

Antioxidant activities of the blueberry fruits

Molecules known as antioxidants prevent oxidation in living organisms by decreasing free radicals or by completely eliminate these (Can *et al*., 2015). There are many methods in order to measure the antioxidant capacity in natural products (Okan *et al*., 2013). To measure the antioxidant capacities of blueberries, this study used the Ferric Reducing Antioxidant Capacity (FRAP), the DPPH radical scavenging activity test and the Beta Carotene colour test (β-carotene). High FRAP and β-carotene and low DPPH values indicate a high antioxidant capacity. Final measurements for the three different antioxidant methods (DPPH, FRAP, Beta Carotene) are indicated in Table 3.

Findings from the Table 3 illustrate that the highest antioxidant activity according to all three methods is seen in natural berries. The highest antioxidant activity according to all three methods among the cultivar berries was observed in 'Duke' and in *V. myrtillus* among the natural berries collected in 2013. The total antioxidant capacity values for blueberries for FRAP were found to be between 454.93-3632.96 μmol troloks/100 g, for β-Carotene between 34.23-86.48%, and for DPPH between 1.01-4.78 mg/mL. When all cultivar and natural berries are examined, it is seen that blueberries are an important antioxidant source. Prior *et al*. (1998), have examined the antioxidant capacities of natural and cultivar blueberries collected in the Oregon (OR), New Jersey (NJ) and Michigan (MI) according to the ORAC (Oxygen Radical Absorbance Capacity) method. The ORAC value of *V. myrtillus* was determined to be 44.6 μmol TE/g according to this study. The other values in this study were reported to be 17.0 μmol TE/g for 'Bluecrop', 18.1 μmol TE/g for 'Jersey' (OR), 20.8 μmol TE/g for 'Jersey' (MI) and 21.4 μmol TE/g for 'Jersey' (NJ) cultivar varieties. Nevertheless, the ORAC capacity of 'Duke' was determined to be 25.1 μmol TE/g and the ORAC capacity of 'O'Neil' was determined to be 16.8 μmol TE/g. From these values, it is seen that the antioxidant capacities of natural berries are much higher than those of cultivars. Furthermore, researchers have noted that regional differences can influence the antioxidant capacities of blueberries of the same variety (Prior *et al*., 1998). In a

Table 3. Antioxidant activity for blueberry fruits using three different complementary assays (FRAP, DPPH, β-Carotene)

Sample	DPPH-SC_{50} (mg/mL)	FRAP (μmol troloks/100 g)	β-Carotene Linoleic Acid (%)
Berkeley (Kaşüstü)	4.07±0.12^{klm}	1140.73±15.28^{gh}	66.40±2.78^{jklm}
Bluecrop (Bulancak)	5.65±0.07^{o}	771.03±18.85^{b}	40.66±2.48^{b}
Bluecrop (Rize)	4.08±0.01^{klm}	454.93±5.58^{a}	66.41±0.77^{jklm}
Bluegold (Bulancak)	3.39±0.05^{i}	1445.87±36.94^{kl}	64.63±0.77^{ijk}
Bluegold (Hayrat)	2.82±0.04^{gh}	1494.55±33.21^{lm}	65.89±2.35^{jkl}
Bluejay (Bulancak)	2.61±0.04^{efg}	1960.16±33.74^{p}	61.51±6.24^{hij}
Bluejay (Kaşüstü)	2.28±0.17^{d}	1814.20±60.09^{o}	62.54±2.78^{hijk}
Blueray (Kaşüstü)	3.84±0.09^{ijk}	985.79±13.89^{e}	49.42±1.54^{e}
Brigitta (Bulancak)	3.72±0.31^{ij}	1067.70±9.69^{fg}	51.48±3.21^{ef}
Brigitta (Hayrat)	3.92±0.4^{kl}	1189.74±23.33^{gh}	62.55±2.54^{hijk}
Chandler (Bulancak)	3.43±0.12^{i}	903.51±12.86^{cd}	46.59±3.65^{cde}
Darrow (Bulancak)	2.75±0.11^{fgh}	1541.02±11.68^{mn}	39.38±3.09^{b}
Duke (Kaşüstü)	1.71±0.05^{c}	2245.15±125.14^{r}	76.06±1.16^{o}
Early Blue (Rize)	4.22±0.08^{m}	954.46±24.12^{de}	48.90±3.80^{de}
Herbert (Kaşüstü)	2.56±0.18^{ef}	1471.12±18.69^{klm}	70.52±3.48^{lmn}
Jersey (Hayrat)	4.16±0.07^{lm}	1006.90±10.64^{ef}	59.97±1.61^{ghi}
Jubile (Bulancak)	3.34±0.22^{i}	1324.99±15.49^{i}	55.60±8.35^{fg}
Legassi (Kaşüstü)	2.74±0.19^{fgh}	1099.17±33.22^{gh}	44.01±2.78^{bcd}
Misty (Bulancak)	2.86±0.03^{h}	828.89±23.11^{bc}	66.92±1.94^{jklm}
Northcountry(Kaşüstü)	2.95±0.15^{h}	1336.77±40.77^{ij}	55.60±0.01^{fg}
Northland (Bulancak)	3.45±0.02^{i}	1095.27±15.30^{gh}	55.59±2.32^{fg}
Oneil (Bulancak)	2.97±0.14^{h}	980.36±13.77^{de}	34.23±4.65^{a}
Ozarkblue (Bulancak)	2.56±0.07^{ef}	1274.26±28.72^{i}	74.90±0.77^{no}
Patriot (Kaşüstü)	4.78±0.05^{n}	1287.16±64.59^{i}	55.34±2.23^{fg}
Puru (Kaşüstü)	2.97±0.07^{h}	1167.27±64.55^{h}	58.42±1.17^{gh}
Putte Sampling	2.80±0.08^{fgh}	1517.64±65.40^{lm}	67.18±0.77^{klm}
Spartan (Kaşüstü)	2.21±0.11^{d}	1611.94±29.90^{n}	51.73±2.78^{ef}
Sunrise (Bulancak)	2.40±0.05^{de}	1294.77±19.03^{i}	71.56±3.56^{mno}
Sunshine (Bulancak)	3.27±0.04^{i}	1280.71±75.33^{i}	59.46±0.01^{ghi}
Toro (Hayrat)	3.94±0.21^{jkl}	1074.60±16.45^{fg}	39.63±5.47^{b}
Toro (Kaşüstü)	4.23±0.16^{m}	935.93±67.78^{de}	55.60±2.32^{fg}
V. arctostaplylos (2012)	1.52±0.02^{bc}	2194.36±25.04^{r}	71.81±2.04^{mno}
V. arctostaplylos (2013)	1.10±0.04^{a}	3080.41±24.02^{t}	73.87±3.12^{no}
V. myrtillus(2012)	1.48±0.07^{b}	2830.73±11.52^{s}	76.71±1.18^{o}
V. myrtillus (2013)	1.01±0.02^{a}	3632.96±82.25^{u}	86.48±0.77^{p}

Different letters (a–z) in the same columns are significantly different at the 5% level ($p < 0.05$). Std. Troloks* 0,008±0,0001; BHT %100

different study the antioxidant capacities of the hybrids 'Northblue', 'Northsky', 'Northcoutry' of *V. angustifolium* × *corymbosum* (VAAC) and the hybrids 'Bluerop', 'Bluejay' and 'Jersey' of the variety *V. corymbosum* (VACM) were examined using the ORAC and FRAP methods. The results showed that the ORAC and FRAP values for 'Northblue' were 26.0 and 26.1 μmol troloks/g, 34.2 and 39.9 μmol troloks/g for 'Northcountry', and 31.3 and 30.5 μmol troloks/g for 'Northsky'. On the other hand, it is noted that the ORAC and FRAP values for 'Bluecrop' were 22.1 and 20.2 μmol troloks/g, 20.7 and 25.5 μmol troloks/g for 'Bluejay', 21.5 and 18.9 μmol troloks/g for 'Jersey'. In the same study, the antioxidant capacities of the hybrids *V. deliciosum* (VADE), *V. membranaceum* (VAME), *V. ovalifolium* (VAOF), *V. ovatum* (VAOV), *V. oxycoccus* (VAOX), *V. parvifolium* (VAPA) and *V. ulignosum* (VAUG) were examined. The mean ORAC and FRAP values of the hybrids were determined as follows: 30.5 and 32.2 μmol troloks/g for VAAC, 21.4 and 21.5 μmol troloks/g for VACM, 14.6 and 30.2 μmol troloks/g for VADE, 21.0 and 40.5 μmol troloks/g for VAME, 37.8 and 76.2 μmol troloks/g for VAOF, 41.1 and 70.2 μmol troloks/g for VAOV, 13.5 and 25.8 μmol troloks/g for VAOX, 7.3 and 10.0 μmol troloks/g for VAPA and 29.3 and 26.1 μmol troloks/g for VAUG (Taruscio *et al*., 2004). In this study, the FRAP values of 'Northcountry' and 'Bluecrop' were lower, 'Bluejay' and 'Jersey' were similar findings compared with literature (Taruscio *et al*., 2004). The mean FRAP value was 14.44 μmol troloks/g and this value was found to be lower than that of all other varieties except VAPA with literature study (Taruscio *et al*., 2004). It was reported in the results of another study, where the antioxidant capacities of the high northern and southern

hybrid blueberries were examined by the ORAC method that the antioxidant capacity was in a range between 4.6 and 31.11 µmol troloks/g. It was found that the antioxidant capacities of 'Berkeley' (5.5 µmol troloks/g), 'Darrow' (14.8 µmol troloks/g), 'Duke' (16.1 µmol troloks/g), 'Spartan' (14.11 µmol troloks/g) and 'Sunshine' (11.7 µmol troloks/g) were lower. The antioxidant capacity of the remaining 'Bluecrop' (10.4 µmol troloks/g), 'Bluegold' (14.9 µmol troloks/g), 'Brigitta' (17.7 µmol troloks/g), 'Chandler' (17.8 µmol troloks/g), 'Herbert' (19.7 µmol troloks/g), 'Jersey' (19.3 µmol troloks/g), 'Jubile' (15.5 µmol troloks/g), 'Oneil' (14.1 µmol troloks/g), 'Legasi' (13.5 µmol troloks/g), 'Misty' (13.9 µmol troloks/g), 'Northland' (17.2 µmol troloks/g), 'Ozarkblue' (17.0 µmol troloks/g) , 'Puru' (22.1 µmol troloks/g) and 'Torro' (19.8 µmol troloks/g) were found to be higher than those in this study. However, these differences are not significant with the exception of 'Toro', 'Bluejay', 'Chandler', 'Earliblue', 'Oneil' and 'Puru' (Ehlenfeldt and Prior, 2001). A study conducted in Italy analyzed the antioxidant capacities of *V. myrtillus* blueberries using the FRAP method and found that the antioxidant capacities were higher in *V. myrtillus* than those of hybrid berries ('Goldtrauble', 'Patriot', 'Bluecrop', 'Darrow'). In this aspect, it shows a similarity with this study. However, the antioxidant capacity of the hybrid and natural berries was found to be much higher than in this study. This is due to the fact that the berry contents and types vary depending on the climate, irrigation, altitude and geographical conditions (Akerström *et al*., 2010; Ribera *et al*., 2010; Ehret, 2012).

Phenolic compounds of blueberry fruits

Phenolics are large group of compounds found many in plants. These compounds inhibit free radicals, which are generated as a waste of the cell metabolism in the human body, and prevent DNA deformation. In particular, phenolic acids like chlorogenic acid and vanillin are major the compounds among the phenolic compounds with the highest radicals scavenge effect (Sawa *et al*., 1999). Blueberries compounds were analysed with HPLC-DAD and these findings are illustrated in Table 4. The analysis was conducted with seventeen standard phenolic compounds and chlorogenic acid was found to be the dominant compound among all of the berries. The compounds gallic acid, protochatecuic acid, *p-OH* benzoic acid, vanillic acid, ellagic acid, rosmarinic acid, o-coumaric acid and curcumin were not found in any of the berries. Compounds such as caffeic acid, syringic acid, t-cinnamic acid, ferulic acid and benzoic acid were found at different levels in some berries. The amount of chlorogenic acid found in the natural berries was higher than the one of the cultivars. The amount of phenolic compounds in the natural berries was much higher than in the cultivar varieties. In addition to this, when the berries of the same variety were obtained from different regions, there were significant differences between the amounts of phenolic compounds found in 'Bluecrop' obtained from Kaşüstü and Bulancak, 'Bluegold' obtained from Rize and Bulancak, 'Bluejay' obtained from Bulancak and Kaşüstü. However, the difference in the amount of phenolic compounds found in the 'Brigitta' samples collected from the Bulancak and Hayrat regions and Toro samples from the Hayrat and Kaşüstü regions is quite high. When the *V. arctostaplylos* and *V. myrtillus* samples obtained in 2012 and 2013 were examined, similar compounds were revealed for both varieties. While quercetin was not found in 2012 in V. *arctostaplylos,* this compound was found in 2013. Similarly, while *p*-coumaric acid was not found in 2012 in *V. myrtillus*, it was found in 2013. Otherwise, the amounts of phenolic compounds found in both years are different. For example, while the amount of chlorogenic acid found in *V. arctostaplylos* in 2012 was 45.6 mg/100 g, in 2013 it decreased to17.66 mg/100 g.

Zimmer *et al*. (2014), reported as a result of the qualitative analysis they performed on hybrid blueberries ('Briteblue', 'Bluegem' and 'Woodard') that chlorogenic acid is the most dominant compound. Authors also found low amounts of quercetin and caffeic acid except in these two compounds (Zimmer *et al*., 2014). The literature is largely in parallel with this study and quercetin is found in the existing study only in some fruit varieties.

Another study conducted in China examined the HPLC and the phenolic compounds of Lanfeng blueberries. The researchers determined that ferulic acid was the most dominant compound among the phenols in berries with 1.280 g kg^{-1}(dry weight). Furthermore, caffeic acid was determined to be the second most dominant compound with 1.217 g kg^{-1}(dry weight). Additionally, the study examined *p*-coumaric acid (1.154 g kg^{-1}(dry weight), syringic acid (0.997 g kg^{-1}(dry weight), vanillic acid (0.170 g kg^{-1}(dry weight) and gallic acid (0.142 g kg^{-1}(dry weight). However, the study did not determine the dry weight of quercetin and kaempferol (Yang *et al*., 2014). Compared with this study, gallic acid and vanillic acid were not found in any blueberry variety. Ferulic acid was determined in 'Bluecrop', 'Blueray', 'Brigitta', 'Darrow', 'Misty', 'Oneil', 'Sunshine' and '*V.myrtillus*' caffeic acid and syringic acid were found in most of the blueberry varieties. However, quercetin was determined in a small amounts of blueberries. According to Häkkinen (2000), the reason for this is that the hybrids of the same plant variety demonstrate differences in the synthesis of the phenolic compounds (Häkkinen, 2000). In a study conducted on the 'Duke' hybrid of *V. corymbosum,* HPLC-DAD analysis on plant varieties demonstrated that the largest phenolic compound was chlorogenic acid. The amount of chlorogenic acid in 'Duke' was determined as 25.42 mg/100 g, 1.59 mg/100 g quercetin, and 1.02 mg/100 g kaempferol (Zheng *et al*., 2003). Similar results were found in this study and it was seen that the most dominant compound was chlorogenic acid. However, quercetin was not found. Previous studies conducted on blueberries illustrated that quercetin was much more prevalent than myricetin and kaempferol. Moreover, ferulic acid was found to be the main compound in blueberries (Stör and Hermann, 1975). Similar results were found in a study conducted by Häkkinen (2000). They also reported in this study that there was no ferulic acid in any of the blueberries. Researcher explained that this was because the peaks of unknown hydroxycinnamate compounds could not be observed due to the dilution of the ferulic acid in the blueberry samples. As a result of this, the peak of ferulic acid disappeared. In addition, the determined that the amount of caffeic acid in 'Northcountry' was very

Table 4. The phenolic compound results of blueberry fruits with HPLC-DAD (mean ±SD measured as mg/100 g sample)

Cultivar	Chlorogenic Acid	Caffeic Acid	Syringic Acid	Ferulic Acid	Rutin	Benzoic Acid	Quercetin	*p*-Coumaric Acid
Berkeley (Kaşüstü)	**2.16±0.27**	N.D.	N.D.	N.D.	**0.39±0.08**	N.D.	N.D.	N.D.
Bluecrop (Bulancak)	**2.87±0.07**	**0.03±0.01**	N.D.	N.D.	**0.71±0.04**	N.D.	N.D.	N.D.
Bluecrop (Rize)	**2.36±1.42**	**0.07±0.02**	N.D.	**0.01±0.02**	**0.59±0.09**	N.D.	N.D.	N.D.
Bluegold (Bulancak)	**3.49±0.76**	**0.37±0.01**	**0.10±0.04**	N.D.	**0.53±0.03**	N.D.	N.D.	N.D.
Bluegold (Hayrat)	**4.15±0.3**	**0.20±0.05**	**0.13±0.01**	N.D.	**0.59±0.14**	N.D.	N.D.	N.D.
Bluejay (Bulancak)	**0.97±0.02**	N.D.	**0.21±0.03**	N.D.	**0.22±0.03**	N.D.	N.D.	N.D.
Bluejay (Kaşüstü)	**1.31±0.1**	N.D.	**0.26±0.05**	N.D.	**0.51±0.06**	N.D.	N.D.	N.D.
Blueray (Kaşüstü)	**4.82±0.07**	**0.002±0.06**	**0.009±0.05**	**0.0031±0.01**	**0.60±0.2**	N.D.	N.D.	N.D.
Brigitta (Bulancak)	**2.13±0.2**	N.D.	**0.29±0.09**	N.D.	**1.27±0.1**	N.D.	N.D.	N.D.
Brigitta (Hayrat)	**8.26±1.19**	N.D.	**0.67±0.12**	**0.0025±0.003**	**0.93±0.08**	N.D.	N.D.	N.D.
Chandler (Bulancak)	**3.97±0.02**	N.D.	N.D.	N.D.	**0.0046±0.05**	N.D.	N.D.	N.D.
Darrow (Bulancak)	**3.05±0.22**	N.D.	N.D.	**0.0150±0.021**	**0.69±0.01**	N.D.	N.D.	N.D.
Duke (Kaşüstü)	**7.58±0.03**	N.D.	N.D.	N.D.	**0.65±0.30**	N.D.	N.D.	N.D.
Early Blue (Rize)	**2.54±0.62**	N.D.	**0.30±0.07**	N.D.	**1.11±0.13**	N.D.	**0.44±0.07**	N.D.
Herbert (Kaşüstü)	**3.65±0.95**	**3.16±0.4**	N.D.	N.D.	**0.72±0.04**	N.D.	N.D.	N.D.
Jersey (Hayrat)	**2.97±0.07**	N.D.	N.D.	N.D.	**0.58±0.09**	N.D.	N.D.	N.D.
Jubile (Bulancak)	**4.01±0.05**	**0.92±0.04**	N.D.	N.D.	N.D.	**0.44±0.06**	N.D.	N.D.
Legasi (Kaşüstü)	**10.68±0.66**	**0.0091±0.002**	N.D.	N.D.	**0.31±0.05**	N.D.	N.D.	N.D.
Misty (Bulancak)	**4.07±0.12**	**0.0061±0.008**	**0.0033±0.04**	**0.0045±0.02**	**1.52±0.04**	N.D.	N.D.	N.D.
Northcountry (Kaşüstü)	**2.76±0.11**	**0.069±0.09**	N.D.	N.D.	**0.56±0.13**	N.D.	N.D.	N.D.
Northland (Bulancak)	**0.50±0.05**	N.D.	**0.46±0.12**	N.D.	**0.54±0.05**	N.D.	N.D.	N.D.
Oneil (Bulancak)	**2.49±0.17**	N.D.	**0.67±0.33**	**0.0031±0.002**	**1.32±0.01**	N.D.	N.D.	N.D.
Patriot (Kaşüstü)	**4.9±3.22**	N.D.	N.D.	N.D.	**0.63±0.3**	N.D.	N.D.	N.D.
Puru (Kaşüstü)	**10.36±4.1**	N.D.	N.D.	N.D.	**1.11±1.23**	N.D.	N.D.	N.D.
Putte Sampling	**2.69±0.76**	N.D.	N.D.	N.D.	**2.73±1.64**	N.D.	N.D.	N.D.
Spartan (Kaşüstü)	**2.95±1.5**	N.D.	N.D.	N.D.	**0.27±0.1**	N.D.	N.D.	N.D.
Sunrise (Bulancak)	**9.94±1.83**	**1.23±0.02**	N.D.	N.D.	**0.86±0.15**	N.D.	N.D.	N.D.
Sunshine (Bulancak)	**8.83±0.25**	**1.95±0.14**	N.D.	**0.046±0.04**	**0.71±0.36**	N.D.	N.D.	N.D.
Toro (Hayrat)	**1.79±0.11**	N.D.	N.D.	N.D.	**0.11±0.01**	N.D.	N.D.	N.D.
Toro (Kaşüstü)	**0.6±0.02**	N.D.	**0.11±0.08**	N.D.	**0.20±0.7**	N.D	**0.02±0.01**	N.D
V. arctostaplylos (2012)	**45.6±7.79**	**0.84±0.1**	N.D.	N.D.	N.D.	N.D.	N.D.	**0.0051±0.003**
V. arctostaplylos (2013)	**17.66±2.77**	**0.28±0.07**	N.D.	N.D.	N.D.	N.D.	**0.07±0.02**	**0.61±0.28**
V. myrtillus (2012)	**15.74±0.33**	**1.96±0.22**	N.D.	**0.20±0.08**	N.D.	N.D.	N.D.	N.D.
V. myrtillus (2013)	**30.13±8.47**	**0.4±0.21**	N.D.	N.D.	N.D.	N.D.	N.D.	**0.55±0.08**

Gallic acid, protocatechuic acid,p-OH benzoic Acid, Vanilic Asid, Ellagic Asit, Benzoic Asid, Rosmarinic Asid,o-Coumaric Asid,t-Cinnamic Asid,Curcumin couldn't be determined.

low and, along with this, that the main phenolic compound of the fruits of *V. myrtillus* was *p*-coumaric acid (Häkkinen, 2000). A similar situation is valid for this study, too, and much less amounts of caffeic acid were found in the 'Northcountry' hybrid. The dominant compound after chlorogenic acid in *V. myrtillus* berries was *p*-coumaric acid. It was reported by Ribera *et al*. (2010) conducted on blueberries that chlorogenic acid and rutin were the dominant compounds in fruits. In addition, compounds such as gallic acid, caffeic acid and ferulic acid were found. The amounts of these compounds were found to be quite low compared with chlorogenic acid and rutin (Ribera *et al*., 2010). Zheng and Wang (2003), reported in the results of their study conducted on the *Vaccinium corymbosum* hybrid that chlorogenic acid was the most dominant phenolic acid with 64.59 mg/100 g. However, in contradiction to the literature, no vanillic acid, caffeic acid and derivates, *p*-coumaric acid and kaempferol were determined (Zheng and Wang, 2003). There is a similar situation with Zheng and Wang (2003) compared with this study and it was determined that the amount of chlorogenic acid was quite low.

Determination of the sugar amounts of blueberry fruits

Sugar compounds are an important psychological process used for determining the quality of sweet fruits. Among these compounds, fructose is especially significant (Kafkas *et al*., 2008). Sugar compounds and amounts are indicated in Table 5. According to this, fructose and glucose sugar were determined as the major monosaccharide in blueberries. In addition to these sugar compounds, sucrose was also found in a large majority of blueberries. Except these, no other sugars were found in any berry variety. While the highest fructose ratio among the berries was identified in 'Patriot', the highest glucose ratio was found in 'Darrow' and 'Duke'. The highest sucrose ratio was found in 'Toro'. The results reveal that the sugar ratios in the others berries varieties were close to each other.

Glycose, fructose, sucrose and malt-sugar were found in the fruit juices as a result of the study conducted on the sugar compounds of blueberry fruit juices made of *V. corymbosum* hybrids. The findings report 3.1 mg/100 g glucose, 4.1 mg/100 g fructose, 0.4 mg /100 g sucrose and 0.5 mg/100 g malt-sugar. Beside this, the authors have stated that the amounts of fructose and glucose were close to each other (Nindo *et al*., 2005). Fructose, glucose and sucrose compounds were found in the fully ripened fruits as a result of the sugar analysis of *V. arctostaphylos* and *V. myrtillus* collected in the Black Sea Region. It was reported that the compounds found in *V. arctostaphylo*s were 25.32% fructose, 26.20% glucose and 1.02% sucrose. These ratios are reported for *V. myrtillus* as 32.90% fructose and glucose and 1.81% sucrose. Briefly, the amounts of the sugar compounds found in *V. arctostaphylos* were lower than those in *V. myrtillus*. Furthermore, the fructose and glucose ratios determined in these berries were very close (Ayaz *et al*., 2001). In another study conducted by Hirvi and Honkanen (1983), on *Vaccinium corymbosum* hybrids and *Vaccinium uliginosum*, fructose and glucose were found in all berries. The fructose amount of the hybrid berries varied between 29-71 g/kg, while the glucose amounts varied between 27-69 g/kg (Hirvi and Honkanen, 1983).

Table 5. Sugar analysis results of the blueberry fruits (mean ±SD measured as mg/100 g sample)

Sample	Fructose	Glucose	Sucrose
Berkeley (Kaşüstü)	6.06±0.05	6.61±0.11	2.93±0.11
Bluecrop (Bulancak)	10.55±0.25	10.24±0.20	3.11±0.10
Bluegold (Bulancak)	8.34±0.08	8.76±0.28	N.D.
Bluejay (Bulancak	9.84±0.17	10.33±0.08	3.51±0.06
Blueray (Kaşüstü)	8.10±0.06	7.98±0.36	2.70±0.04
Brigitta (Bulancak)	9.44±0.07	11.92±0.10	N.D.
Chandler (Bulancak)	12.14±0.18	10.96±0.14	3.01±0.02
Darrow (Bulancak)	12.07±0.18	12.85±0.37	2.90±0.40
Duke (Kaşüstü)	11.73±0.13	12.03±0.35	3.40±0.04
Early Blue (Rize)	7.82±0.55	7.58±0.31	N.D.
Herbert (Kaşüstü)	10.55±0.25	10.24±0.20	3.11±0.10
Jersey (Hayrat)	11.15±0.12	10.91±0.24	3.07±0.16
Jubile (Bulancak)	9.12±0.37	8.90±0.28	2.80±0.05
Legasi (Kaşüstü)	7.97±0.15	8.45±0.24	2.90±0.11
Misty (Bulancak)	9.56±0.31	8.16±0.36	2.60±0.14
Northcountry (Kaşüstü)	9.11±0.12	9.06±0.12	1.23±0.05
Northblue (Bulancak)	4.99±0.06	6.07±0.03	2.92±0.04
Northland (Bulancak)	12.11±0.17	10.35±0.04	2.61±0.04
Oneil (Bulancak)	9.42±0.03	8.33±0.04	2.62±0.20
Ozarkblue (Bulancak)	6.28±0.12	7.13±0.14	2.74±0.06
Patriot (Kaşüstü)	13.74±0.05	11.14±0.03	1.20±0.06
Puru (Kaşüstü)	11.83±0.12	11.05±0.10	3.33±0.11
Putte Sampling (Kaşüstü)	7.54±0.44	8.32±0.17	2.81±0.20
Spartan (Kaşüstü)	8.75±0.13	9.18±0.05	3.82±0.40
Sunrise (Bulancak)	9.63±0.35	8.63±0.04	N.D.
Sunshine (Bulancak)	4.73±0.02	4.44±0.08	N.D.
Toro (Hayrat)	10.83±0.10	10.96±0.51	3.51±0.30
V. arctostaplylos	5.96±0.15	5.17±0.06	N.D.
V. myrtillus	7.95±0.22	8.05±0.09	N.D.

Ribose, Arabinose, Xsylose, Malt-Sugar,Theralose, Melabiose and Melezitose couldn't be determined.

Table 6. Correlation results of the analysis performed at blueberry fruits

	TPC	TFC	TAC	DPPH	FRAP	β-Carotene
TPC	**1**	0.464	0.588	0.713	0.765	0.709
TFC	0.464	**1**	0.826	0.546	0.556	0.499
TAC	0.588	0.826	**1**	0.676	0.729	0.526
DPPH	0.713	0.546	0.676	**1**	0.932	0.627
FRAP	0.765	0.556	0.729	0.932	**1**	0.613
β-Carotene	0.709	0.499	0.526	0.627	0.613	**1**

Correlation at 0.01 level is important.

Correlation analysis for blueberries

This study used the Pearson Correlation Tests to determine the relation of total polyphenol, flavonoid, anthocyanin, DPPH, FRAP and β-Carotene analysis in the blueberries. The results are shown in Table 6.

While a medium correlation was determined between polyphenol and flavonoid, there was a high correlation between polyphenol and anthocyanin. Also, there was a high correlation between FRAP, one of the antioxidant methods, β-carotene and DPPH and polyphenol. Among these was a highly correlation between DPPH and polyphenol. While there was a slight correlation between FRAP values and β-carotene, a slightly correlation was determined between DPPH and flavonoid. There was a strong positive correlation between flavonoid and anthocyanin. The Pearson Correlation revealed that there was a high positive correlation between anthocyanin and FRAP, a medium correlation between anthocyanin and β-carotene, a medium correlation between anthocyanin and DPPH. A highly correlation was found between DPPH, FRAP and β-carotene.

Many of the studies conducted on food have shown that the total polyphenol and total anthocyanin amounts have an important effect on antioxidant capacity. This has been proven in the majority of studies by the high correlation between the total amount of polyphenols and the total amount of anthocyanin (Uzelac *et al*., 2009). At the result of the study, where the correlation between the antioxidant (ORAC), total phenol and anthocyanin results of the blueberry fruits is examined, there is a low but meaningful correlation between the phenolic amount and the anthocyanin amount determined in blueberries (Ehlenfeldt and Prior, 2001). Connor et al. (2002) found a high correlation between the antioxidant activity and the total polyphenol compounds (r=0,88). Besides, they also found a low correlation between anthocyanin and antioxidants (r=0,61) (Connor *et al*., 2002). Additionally, there was no correlation between the phenolic compounds and antioxidants and total phenolic amounts (r=0,30). However, there was a strong correlation between the flavonol amounts and the antioxidant activity (r=0,78) (Kahkönen *et al*., 2001). The total polyphenol, total flavonoid and total antioxidant amounts of different blueberry hybrids were examined by 4 different antioxidant methods (FRAP, ABTS, ORAC and DPPH). It was reported that there was a high correlation between the antioxidant methods (Bunea *et al*., 2011).

Conclusions

Our findings reveal that the total polyphenol, total anthocyanin, total flavonoid and antioxidant contents of natural blueberries were higher compared with those of the cultivars. Furthermore, blueberries are a high antioxidant source. As a result of the HPLC-DAD analysis conducted on blueberries, our findings demonstrate that the dominant compound was chlorogenic acid. Fructose and glucose were found in all berries as result of the sugar analysis. In addition to this, sucrose was found in a great majority of the berries. However, the amount of sucrose was lower than fructose and glucose. There were quite significant differences observed with regards to phenolic and antioxidant attributes between the samples of the same berry variety collected from different regions and the same berry variety collected from the same regions during different years. It is thought that the reason for this is the genotype and climatic differences between the hybrids. While a medium correlation was found between the total polyphenol, total flavonoid, total anthocyanin contents, there was a strong correlation between the antioxidant results of the different methods (FRAP, DPPH, β-Carotene).

The data shows cultivars are also comparable with naturally grown species (low bush blue berry native to Turkey) regarding bioactivity as well as composition. According to findings the northern highbush species are the most suitable species for the Black Sea region.

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