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Influence of hot air drying on phenolic compounds and antioxidant capacity of blueberry (*Vaccinium myrtillus)* fruit and leaf

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Summary

The present study was undertaken to assess the effects of hot air drying on phenolic compositions, total phenolic (TP) content, total anthocyanin (TA) content, as well as antioxidant capacities of methanol extracts from blueberry (*Vaccinium myrtillus)* fruit and leaf introduced in Kapıdağ region of Turkey climate conditions. A total of twenty-two phenolic standards were screened by HPLC, total phenols were measured by spectrophotometric methods, antioxidant capacity was determined using DPPH, CUPRAC, ABTS, and FRAP assays in the blueberry fruit and leaf extracts. Analysis by HPLC revealed that fruit extracts have different phenolic profiles due to drying process and contain syringic acid, myricetin, naringin, (-)-epicatechin, and malvidine-3-*O*-glucoside chloride as the main compounds. Leaf extracts had higher resveratrol concentrations than fruit extracts. The TP and TA contents gradually increased when the blueberry fruits were dried under hot air condition. The fresh and dried blueberry fruit and leaf extracts showed similar antioxidant capacity values. Significant relationships between antioxidant capacity and TP were found.

Keywords *Vac. myrtillus*; blueberry; hot ait drying; fruit; leaf; phenolics; antioxidant capacity

Introduction

Blueberry, a perennial shrub of the genus *Vaccinium*, family Ericaceae, became well known around the world due to high levels of phenolic compounds (Ehlenfeldt and Prior, 2001; Prior et al., 2001; Kim et al., 2010; Routray et al., 2014). These compounds have been reported to have numerous valuable health benefits including superb antioxidant, anti-hypertensive, anti-diabetic, anti-leukemia, anti-obesity, anti-inflammatory, and anti-microbial activity, as well as neuroactive properties, to protect against cancer and stroke (Ehlenfeldt and Prior, 2001; Deng et al., 2014; Li et al., 2013). Blueberries are considered to be one of the richest sources of phenolic compounds and antioxidant phytochemicals among fruits and vegetables, and they contain significant levels of anthocyanins, flavonols, flavonons, proanthocyanidins, and phenolic acids (Castrejón et al., 2008; Wang et al., 2012). Factors that have an impact on the total phenolic content, total anthocyanins, and the antioxidant capacity of blueberries fruit and leaves, include genetic differences, the cultivar type, growing location and season, agronomic factors, the degree of maturity at harvest, and postharvest storage conditions (Ehlenfeldt and Prior, 2001; Deng et al., 2014).

Drying, as a preservation method, is a very important aspect of food processing. The main functions of drying are lowering the water activity, inhibiting the growth of microorganisms, decreasing chemical reactions, extending shelf life, allowing for room temperature storage, reducing transportation costs with regard to refrigeration, and also enhancing visual and taste of cereals, confections, and baked goods. Several drying techniques such as sun drying, convection oven drying, freeze drying, microwave drying etc., have been employed in an effort to achieve high quality dried blueberries (Mejia-Meza et al., 2008; Hamrouni-Sellami et al., 2013). The selection of drying methods to be used is dependent on the use of the end product, economic viability, availability of resources, and composition of the biomaterial (Routray et al., 2014). Convective hot air drying is a traditional, low cost technique that is widely used to lower the water content of fresh products at present, nevertheless it requires relatively long times and high temperatures, causes degradation of important nutrients, has shrunken and toughened dried products with noticeable browning, and allows for little rehydration ability (Sellappan et al., 2002).

The aim of this study was to determine the influence of location, part of plant (fruit or leaf), and oven drying on the phenolic compounds, total phenol and anthocyanin contents, and antioxidant capacities of blueberries (*Vac. myrtillus*).

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Material and methods

Sample collection and preparation

Blueberry leaves and fruit grown at three different locations of Erdek (sea level, Balikesir, Turkey) and Kapıdağ (altitudes of 650 m, Balıkesir, Turkey) regions were randomly hand-picked during October and November, 2014, and transported to the laboratory within the same day. Upon arrival, the fruit and leaves were hand selected and separated into two lots of equal weight. One lot of fruit and leaves were stored separately fresh, and the other lots were firstly dried at 50 °C for 2 h in an air oven type FN 055 (NUVE, İstanbul, Turkey) then cooled. The two lots were then separately vacuum packaged (VC 999/K12NA packing machine, Verpackungssysteme AG, Herisau, Switzerland) in FMXBK polyamide-polyethylene film (PO_2=15 cm^3/m^2/24 h at 23 °C and 75% relative humidity; Flexopack S.A. Plastics Industry, Koropi, Greece) and stored at -20 °C (SF 312, Dairei, Tokyo, Japan) until further analysis. Extracts of *Vac. myrtillus* fruit and leaf were prepared according to the method of described by Ehlenfeldt and Prior (2001) and Prior et al. (2001), with some modifications. Fresh and dried fruit and leave samples (2 g) were separately extracted twice with 20 mL of methanol:formic acid (99.5/0.5, v/v, for fruit), and acetone:formic acid (99.5/0.5, v/v, for leaf) mixture in an ultrasonic bath at room temperature (20 °C) for 15 min. Extracts then were separately centrifuged at 3500 rpm for 10 min at 4 °C in a centrifuge (Sigma 3K30, UK). The supernatants were combined, after removal of methanol and acetone with a rotary evaporator (Heidolph Laborota 4001, Germany) under vacuum conditions at 40 °C, the residual extracts were subjected to a liquid-liquid partition with methanol:formic acid (99.5/0.5, v/v, for fruit) and acetone:formic acid (99.5/0.5, v/v, for leaves), respectively, filtered through a nylon filter membrane (Sigma Z290793, pore size 0.45 μm, diam. 47 mm), transferred to vials, and stored -20 °C until further analysis.

Chemicals

Phenolic standards were obtained from Fluka (St. Louis, MO, USA) (gallic acid; CAS:149-91-7, ferulic acid; CAS:537-98-4, resveratrol; CAS:501-36-0, (+)-catechin; CAS:154-23-4, (-)-epicatechin; CAS:490-46-0, myricetin; CAS:529-44-2, kaempferol; CAS:520-18-3, (-)-epigallocatechin; CAS:970-84-1, Sigma (St. Louis, MO, USA) (quercetin; CAS:117-39-5, caffeic acid; CAS:331-39-5, syringic acid; CAS:530-87-4, *p*-coumaric acid; CAS:501-98-4, naringin; CAS:10236-47-2, hesperidin; CAS:520-26-3, neohesperidin; CAS:13241-33-3, rutin hydrate; CAS:207671-50-9, cyanidin-3-*O*-glycoside chloride; CAS:7084-24-4, malvidine-3-*O*-glycoside chloride; CAS:7228-78-6), Aldrich (St. Louis, MO, USA) (vanillic acid; CAS:121-34-6, trans ferulic acid; CAS:537-98-4, 3-hydroxy-4-metoxy-cinnamic acid; CAS:637-73-5), HWI Analytik GmbH (Ruelzheim, Germany) (chlorogenic acid; CAS:327-97-9). Calibration curves were made by diluting stock standards in methanol.

Determination of phenolic composition using HPLC

Phenolic compositions were analysed according to a previously reported method with modifications in HPLC elution conditions (Sellappan et al., 2002). The phenolic extracts, phenolic standards and also all solvents were filtered through a nylon filter membrane (Sigma Z290793, pore size 0.45 μm, diam. 47 mm) prior to HPLC analysis and then analysed in a HPLC chromatography system (Shimadzu Class VP V.6.14 SP1, USA) equipped with Shimadzu Diode Array Detector (SPD-M 10A), VP and reversed-phase C18 column (Zorbax Eclipse XDB, Agilent, 4.6 mm, 150 mm, 5 μm). The temperature of the column oven was set at 40 °C. The wavelengths used for the quantification of phenolic compounds by the detector were: 280 nm for syringic acid, gallic acid, (+)-catechin, neohesperidin, caffeic acid, hesperidin, (-)-epigallocatechin, (-)-epicatechin, naringin, vanillic acid; 320 nm for *trans*-ferulic acid, chlorogenic acid, 3-hydroxy-4-metoxy-cinnamic acid, resveratrol, *p*-coumaric acid, ferulic acid; 360 nm for myricetin, rutin hydrate, kaempferol, quercetin; and 520 nm for cyanidin-3-*O*-glycoside chloride, malvidine-3-*O*-glycoside chloride. A gradient elution was employed with mobil phase consisting of methanol:water:formic acid (3.5/96.4/0.1, v/v/v, solvent A) and acetonitril:formic acid (98/2, v/v, solvent B) as follows: the composition of B was increased from 0.5% to 7.5% after 31 min, increased to 10% for 9 min, and increased to 14% for 5 min, increased to 18% for 5 min, increased to 30% for 10 min, increased to 45% for 5 min, and increased to 60% for 5 min. The composition was decreased to 40% for 5 min. The injection volume was 20 μL, the flow rate was 0.7 mL/min at room temperature, the duration of a single run was 75 min. All phenolic acids were quantified using an external standard. The total phenolic extracts and standard compounds were analyzed under the same analysis conditions and a 10 min equilibrium time was allowed between injections. All standard and sample solutions were injected in triplicate.

Determination of total phenolic (TP) content

The total phenolic (TP) contents of fresh and dried blueberry fruit and leave extracts were measured by the Folin-Ciocalteu method described by Singleton et al. (1999), with some modifications. Briefly, an aliquot (0.5 mL) of appropriately diluted extracts, or standard solutions of gallic acid, 1.5 mL of double distilled water and 2.5 mL Folin-Ciocalteu reagent, were mixed within volumetric flasks at room temperature. After 10 min, 0.25 mL of 7.5% sodium carbonate (1:3 diluted with double distilled water) solution (m/v) was added and mixed thoroughly. The absorbance of the solution was measured using a spectrophotometer (UVMecasys Optizen 3220) at 750 nm after 30 min in the dark at room temperature. Methanol was used as the blank and gallic acid was used for calibration of the standard curve (0-500 mg/L). The results were expressed as mg of gallic acid equivalents (GAE) per kg. Each extract was measured in triplicate.

Determination of total anthocyanin (TA) content

The total anthocyanin content of extracts obtained from blueberry fruit and leaves were determined by means of the pH-differential method as described by Sellappan et al. (2002). The absorbance was measured using a spectrophotometer (UVMecasys Optizen 3220) at 700 nm and at the wavelength of maximum absorption (520 nm) against a blank and calculated as:

$$A = (A_{520} - A_{700})\ pH_{1.0} - (A_{520} - A_{700})\ pH_{4.5}$$

Monomeric anthocyanin pigment concentration of extracts was calculated as cyanidin-3-glucoside equivalent and each extract was measured in triplicate.

$$\text{Monomeric anthocyanin pigment (mg/L)} = A \times MW \times DF \times 1000\ (\varepsilon \times 1)$$

where A = absorbance, MW = molecular weight (449.2), DF = dilution factor, ε = molar absortivity (26900). The final concentration of total anthocyanins (mg/kg) was calculated based on total volume of extract and weight of sample.

Determination of antioxidant capacity by cupric ion reducing antioxidant capacity (CUPRAC) assay

Determination of CUPRAC was conducted according to the method by Apak et al. (2007). One mL 10 mmol/L $CuCl_2$, 1 mL 7.5 mmol/L neocuproine, 1 mL 1 M NH_4Ac, × mL extract, and (4-×) mL H_2O were mixed. The tubes were stopped and after 30 min the final absorbance was recorded using a spectrophotometer (UVMecasys Optizen 3220) at 450 nm against a reagent blank. A standard curve was prepared using different concentrations of Trolox. The calculations of the antioxidant capacity of phenolic antioxidants were expressed as μmol of Trolox equivalent (TE) per gram. Each extract was measured in triplicate.

Determination of antioxidant capacity by DPPH (2,2-diphenyl-2-picrylhydrazyl) free radical assay

The free radical scavenging capacity of the blueberry fruit and leave extracts were determined by colorimetric method described by Brand-Williams et al. (1995). In brief, the appropriately diluted extracts (× mL), methanol (4-× mL), and DPPH solution (3.9 mL, 50 μM) in methanol were incubated in a water bath at 37 °C for 30 min. After incubation, the absorbance was measured at 515 nm with a spectrophotometer (UVMecasys Optizen 3220). The results were calculated against methanol without DPPH and compared to a different concentration of Trolox standard curve. Each extract was measured in triplicate. DPPH values, derived triplicate analyses, were expressed as μmol of Trolox equivalent (TE) per gram and were calculated as follows:

$$\text{DPPH radical scavenging capacity (\%)} = (1-[A_{sample}/A_{control}]) \times 100$$

Determination of antioxidant capacity by ABTS [2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)] assay

The ABTS method is based on the deactivation of the antioxidant radical cation ABTS·+. The ABTS method was performed as described by Re et al. (1999). ABTS radical cation ($ABTS^+$) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) aqueous solution and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Different concentrations of fruit and leaf extracts were mixed with 1 mL of diluted ABTS·+ solution and the reduction of ABTS·+ radical was measured by the decrease in absorbance at 734 nm after 6 min by

using the spectrophotometer UVMecasys Optizen 3220. To develop a standard curve, a standard Trolox solution was diluted with ethanol and added to 1 mL of the diluted ABTS·+ solution. The controls contained the extraction solvent instead of the test samples. Each extract was measured in triplicate. The scavenging capacity of ABTS free radical was calculated as:

ABTS radical scavenging capacity (%) = $(1-[A_{sample}/A_{control}]) \times 100$

Determination of antioxidant capacity by FRAP assay

The FRAP assay was conducted according to Benzie and Strain (1996). This method is based on an increase of the absorbance at 593 nm due to the formation of tripyridyl-*S*-triazine complexes with Fe^{2+} [TPTZ-Fe(II)] in the presence of a reductive agent. The FRAP reagent was prepared by mixing TPTZ solution (10 mmol/L) in hydrochloric acid (40 mmol/L) and $FeCl_3$ solution (20 mmol/L) mixed with 25 mL of acetate buffer (0.3 mol/L, pH=3.6). An appropriately diluted sample extract (× µL) and FRAP reagent (1-× mL) were added and, the mixture and extraction or solvent for the reagent blank were incubated at 37 °C for 30 min. At the end of incubation, absorbance was immediately measured using a spectrophotometer (Perkin Elmer UV/VIS Lambda35) at 595 nm. Solutions of Trolox dissolved in extraction solvent, ranging from 10-100 µmol/L were used for preparation of a calibration curve. FRAP values, derived from triplicate analyses, and were expressed as µmol of Trolox equivalent (TE) per gram. Each extract was measured in triplicate.

Statistical analysis

Statistical differences between the data sets were determined by two-way Analysis of variance (ANOVA) using the SPSS statistical package (SPSS 16.0, Chicago, IL). Differences between treatments that are described subsequently as being significant, were determined at least $p<0.05$. The least significant difference (LSD) test was used to determine differences between means.

Results and discussion

Phenolic compositions

The data set for the contents of phenolic acids, flavonols, flavanones, monomeric of flavan-3-ol derivatives, anthocyanins, and the stilbene in the *Vac. myrtillus* fresh and dried fruit and leaf extracts are given in Tab. 1-4. These compounds can act as antioxidants and may be important components of functional foods. The dominant phenolic acid was syringic acid in fresh fruit extracts grown in Erdek and Kapıdağ regions (28.79-637.43 mg/kg FW, 339.13-995.15 mg/kg FW, respectively), and their content was especially high ($p<0.05$) in dried fruits. In fresh and dried *Vac. myrtillus* leaf extracts, syringic, *p*-coumaric, gallic and vanillic acids were the most abundant phenolic acids. Some research has reported that chlorogenic acid, referred to as 5-*O*-caffeoylquinic acid (5-CQA), is considered a major colourless phenolic acid in blueberry fruit and leaf (Harris et al., 2007; Kim et al., 2010), and a more readily available sources of 5-CQA, even compared to green coffee beans (Kim et al., 2010). Prior et al. (2001) found the level of chlorogenic acid in blueberries to be 60-100 mg/g of fresh fruit, while Harris et al. (2007) detected this compound 30 times more concentrated in the leaf extract than in fruit. Nevertheless, in this study high chlorogenic acid levels in the fruit and leaf extracts were not determined. On the other side, vanillic acid, 3-hydroxy-4-metoxy cinnamic acid, and ferulic acid were obtained in fresh fruit extracts, whereas in dried fruit extracts, higher levels of vanillic acid, gallic acid, *p*-coumaric acid, ferulic acid, and 3-hydroxy-4-metoxy cinnamic acid were determined. Apart from chlorogenic acid, the phenolic acids have been found to be present in smaller concentrations, such as caffeic, *p*-coumaric and ferulic acid (Sellappan et al., 2002). In addition, other phenols that may be found include gallic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, ellagic, vanillic, protocatechuic, gentisic, syringic, sinapic and salicylic acids, and catechin, epicatechin, myricetin, and kaempferol (Sellappan et al., 2002; Harris et al., 2007). In our samples obtained from Erdek and Kapıdağ, we found some of these components, and most phenolic compounds detected in this study were consistent with previous reports on blueberry fruit and leaves from different locations in the world. It is believed that the significant qualitative and quantitative differences ($p<0.05$) of phenolic compounds profiles which have been confirmed in blueberries, are due to variations in genotypes, locations, cultivation conditions, increased maturity, different parts of plants examined, stresses, organically grown, extraction methods, and all can have varying effects on the level of total anthocyanins, total phenolics and antioxidant capacity (Xiaoyong and Luming, 2014). Also drying conditions can cause differences.

Some recent studies have been accomplished on the content of blueberry and bilberry native flavonols (Sellappan et al., 2002; Harris et al., 2007; Moze et al., 2011; Vrhovsek et al., 2012). In this study, fresh *Vac. myrtillus* fruit extracts contained low flavonols contents (0.79-91.98 mg/kg FW), whereas dried fruit and leaf extracts contained relatively high flavonols. While myricetin was found to be the main flavonol compound in the leaves, kaempferol and quercetin were also detected, in agreement with other studies. Previous research also determined that the green leaves of blueberry contained a much larger amount of flavonoids (quercetin and kaempferol) and hydroxycinnamic acid (*p*-coumaric and caffeic acid) than fruits (Riihinen et al., 2008). Nevertheless, in this study the concentrations of myricetin were higher in the fresh leaves extracts than fresh fruit extracts ($p<0.05$). Also in the dried fruit extracts, the concentrations of flavonols were considerably higher than in the dried leaf extracts. As mentioned by Jaakola et al. (2004) and Oszmiański et al. (2011), *p*-coumaric acid is the precursor of flavonoids, and the increase in *p*-coumaric acid concentration in the leaves, growing under high solar radiation, can also reflect the overall activation of flavonoid biosynthesis. Tab. 1-4 shows that all the flavonol compounds had some significant ($p<0.05$) level of variability due to altitude, and due to the drying process. Rutin hydrate, which has been reported in high amounts in buckwheat (Moze et al., 2011), was found in both regions of *Vac. myrtillus* fruits under investigation, the dried fruit extracts containing more rutin hydrate than fresh fruit extracts and also compared to the fresh and dried leaf extracts. Moze et al. (2011) first detected rutin in bilberry and blueberry samples (0.2 and 3.1 mg/100g FW, respectively), and also rutin (quercetin-3-*O*-rutinoside) was the major phenolic compounds in leaves of rabbiteye blueberry cultivated in Japan (Li et al., 2013). Harris et al. (2007) determined this compound as 3.10 mg/100 g in *Vac. corybocum* L. fruits, whereas our results were higher than their results. Bioflavonoids like rutin and naringin have been proven to be efficacious antioxidants and are widely distributed in fruits and vegetables. Rutin belongs to the class of flavonols and naringin belongs to flavanones. It is well noted and proven in several studies, that flavonols are very active in conveying therapeutic benefit compared to flavonones (Akondi et al., 2011). The flavonones (naringin, hesperidin, and neohesperidin) were detected in studied *Vac. myrtillus* extracts. Naringin is one of the most abundant flavanone in fresh and dried fruit and leaf extracts in this study. It was also observed that naringin levels were greater in samples harvested from high altitude compared to those samples originating from areas at sea level. Some reports have indicated that drying methods can affect phenolic contents and the antioxidant capacity of plant materials due to drying time/temperature, light intensity, packaging, and storage time etc. (Lu and Luthria, 2014).

The three flavanols were found in both regions of *Vac. myrtillus*, the dried fruit extracts being a better source of (+)-catechin, (-)-epicate-

Tab. 1: Phenolic compounds concentrations of fresh *Vac. myrtillus* fruits grown in Erdek ve Kapıdağ regions (mg/kg)

Phenolic compounds	E1FRF*	E2FRF	E3FRF	K1FRF	K2FRF	K3FRF
Gallic acid	19.19 ± 2.12^{Cb**}	36.82 ± 4.58^{Aa}	30.29 ± 3.14^{Ba}	10.00 ± 1.13^{Dk}	8.10 ± 1.10^{Dl}	0.87 ± 0.12^{Em}
Vanillic acid	532.97 ± 25.16^{Aa}	52.36 ± 3.25^{Cc}	289.61 ± 5.12^{Bb}	20.91 ± 1.95^{El}	9.25 ± 1.63^{Fm}	29.75 ± 1.98^{Dk}
Caffeic acid	5.46 ± 1.23^{Aa}	4.84 ± 1.10^{Bc}	5.35 ± 1.00^{Ab}	1.87 ±0.12^{Ck}	1.86 ± 0.24^{Ck}	1.73 ± 0.23^{Dl}
Chlorogenic acid	1.25 ± 0.25^{Ec}	4.37 ± 0.85^{Aa}	1.71 ± 0.26^{Cb}	1.42 ± 0.10^{Dl}	1.50 ± 0.27^{CDl}	2.71 ± 0.45^{Bk}
Syringic acid	637.43 ± 3.45^{Ba}	28.79 ± 4.85^{Fc}	32.30 ± 1.26^{Eb}	529.53 ± 6.52^{Cl}	995.15±6.54^{Ak}	339.13± 3.21^{Dm}
***p*-Coumaric acid**	7.63 ± 1.48^{Ca}	1.81 ± 0.12^{Ec}	4.04 ± 1.85^{Db}	11.18 ± 2.13^{Bl}	20.05 ± 2.31^{Ak}	8.68 ± 1.00^{Cm}
Ferulic acid	10.60 ± 2.69^{Da}	6.06 ± 0.95^{Eb}	4.13 ± 0.74^{Fc}	20.54 ± 2.31^{Bl}	24.87 ± 1.98^{Ak}	14.77 ± 1.14^{Cm}
Trans-ferulic acid	5.99 ± 1.78^{Da}	1.89 ± 0.14^{Eb}	5.45 ± 1.23^{Da}	8.49 ± 2.85^{Cm}	11.50 ± 2.14^{Bl}	19.40 ± 1.56^{Ak}
3-hydroxy-4-methoxy cinnamic acid	50.63 ± 4.65^{Db}	75.95 ± 4.65^{Ca}	26.29 ± 2.32^{Ec}	78.11 ± 3.12^{BCm}	103.39 ± 6.54^{Ak}	80.61 ± 3.24^{Bl}
Myricetin	51.64 ± 2.98^{Db}	55.12 ± 3.12^{Da}	43.06 ± 2.96^{Ec}	79.78 ± 2.96^{Cm}	91.98 ± 3.45^{Ak}	83.39 ± 3.59^{Bl}
Quercetin	1.62 ± 0.12^{Ec}	4.27 ± 1.00^{Bb}	6.93 ± 1.85^{Aa}	2.48 ± 0.62^{Dl}	3.71 ± 0.56^{Ck}	2.24 ± 0.46^{Dl}
Kaempferol	1.72 ± 0.45^{ABa}	0.94 ± 0.16^{Db}	1.82 ± 0.15^{Aa}	0.79 ± 0.10^{Em}	1.01 ± 0.15^{Dl}	1.67 ± 0.37^{Bk}
Rutin hydrate	3.84 ± 1.10^{Da}	1.31 ± 0.25^{Fc}	2.07 ± 0.23^{Eb}	13.30 ± 1.10^{Bl}	24.01 ± 1.47^{Ak}	7.64 ± 0.96^{Cm}
Naringin	5.50 ± 1.14^{Dc}	33.60 ± 3.74^{Bb}	79.37 ± 3.41^{Aa}	4.42 ± 0.65^{Em}	6.97 ± 0.84^{Ck}	6.13 ± 0.45^{Dl}
Hesperidin	7.07 ± 1.56^{Cc}	27.45 ± 2.85^{Bb}	31.59 ± 2.16^{Aa}	2.40 ± 0.13^{El}	4.93 ± 0.16^{Dk}	2.35 ± 0.78^{El}
Neohesperidin	2.50 ± 0.45^{Bb}	8.38 ± 2.14^{Aa}	8.80 ± 1.62^{Aa}	1.83 ± 0.14^{CDl}	1.92 ± 0.11^{Ck}	1.73 ± 0.15^{Cbm}
(+)-Catechin	2.72 ± 0.41^{Dc}	29.57 ± 2.48^{Aa}	9.45 ± 1.64^{Bb}	6.41 ± 0.64^{Ck}	1.36 ± 0.12^{Em}	2.63 ± 0.43^{Dl}
(-)-Epicatechin	28.54 ± 3.41^{Da}	10.43 ± 1.96^{Eb}	8.63 ± 1.18^{Fc}	50.63 ± 2.63^{Bl}	80.23 ± 4.85^{Ak}	46.04 ± 2.45^{Cm}
(-)-Epigallocatechin	6.66 ± 1.58^{Dc}	24.99 ± 3.11^{Bb}	82.62 ± 3.98^{Aa}	17.88 ± 1.84^{Cl}	22.79 ± 2.41^{Bk}	16.32 ± 1.47^{Cm}
Cyanidin-3-*O*-glucoside chloride	373.38 ± 15.69^{Ca}	70.94 ± 4.23^{Eb}	384.06 ± 4.85^{Ca}	438.33 ± 5.23^{Bl}	781.26 ± 6.52^{Ak}	335.60 ± 6.21^{Dm}
Malvidine-3-*O*-glucoside chloride	3231.38 ± 25.89^{Ca}	212.93 ± 6.52^{Fc}	329.94 ± 6.12^{Eb}	3644.61 ± 8.95^{Bl}	4433.19 ± 9.96^{Ak}	2433.10 ± 5.27^{Dm}
Resveratrol	1.01 ± 0.05^{Aa}	0.87 ± 0.05^{Bb}	0.78 ± 0.05^{Bb}	0.70 ± 0.06^{Bm}	0.84 ± 0.06^{Bl}	1.00 ± 0.00^{Ak}

* E: Erdek, K: Kapıdağ, FR: Fresh, F: Fruit, 1-3: Codes of samples collected from different regions, ** Mean values (mg/kg)±standard deviation (N=3×2) with different capital letters (A-F) in the same row are significantly different (p<0.05) according to collected from different region at fresh fruit. Mean values±standard deviation (N=3×2) with different lowercase (a-c. k-m) in the same row are significantly different (p<0.05) according to collected from the same region at fresh fruit.

Tab. 2: Phenolic compounds concentrations of dried *Vac. myrtillus* fruits grown in Erdek ve Kapıdağ regions (mg/kg (mg/kg)

Phenolic compounds	E1DRF*	E2DRF	E3DRF	K1DRF	K2DRF	K3DRF
Gallic acid	43.53 ± 4.78^{Cb**}	28.11 ± 1.78^{Ec}	57.63 ± 5.41^{Ba}	52.29 ± 2.18^{Bl}	10.43 ± 1.10^{Dm}	72.59 ± 3.25^{Ak}
Vanillic acid	244.73 ± 3.65^{Bb}	352.64 ± 3.65^{Aa}	123.76 ± 3.29^{Cc}	42.04 ± 2.87^{Em}	117.16 ± 2.85^{Ck}	71.00 ± 4.58^{Dl}
Caffeic acid	3.92 ± 1.85^{Db}	2.19 ± 0.18^{Ec}	6.99 ± 1.23^{Ca}	3.80 ± 0.51^{Dl}	35.14 ± 1.47^{Ak}	31.88± 1.95^{Bm}
Chlorogenic acid	1.19 ± 0.14^{Eb}	0.94 ± 0.02^{Fb}	1.66 ± 0.45^{Da}	2.11 ± 0.11^{Cm}	8.04 ± 1.45^{Ak}	2.86 ± 0.10^{Bl}
Syringic acid	1338. 96 ± 16.84^{Db}	245.49 ± 1.85^{Fc}	2971.61 ± 25.56^{Ca}	3344.54 ± 28.62^{Bl}	5627.47 ± 32.14^{Ak}	304.33 ± 3.85^{Em}
***p*-Coumaric acid**	22.25 ± 2.14^{Eb}	9.09 ± 1.63^{Fc}	73.98 ± 3.45^{Da}	390.96 ± 3.61^{Bl}	557.22 ± 3.25^{Ak}	137.30 ± 4.45^{Cm}
Ferulic acid	11.92 ± 3.16^{Db}	7.49 ± 1.74^{Ec}	24.32 ± 2.89^{Ca}	94.46 ± 3.54^{Ak}	77.55 ± 1.85^{Bl}	71.48 ± 6.18^{Bl}
Trans-ferulic acid	1.54 ± 0.14^{Eb}	1.06 ± 0.16^{Ec}	2.52 ± 0.85^{Da}	20.17 ± 4.52^{Ak}	15.59 ± 3.85^{Cm}	18.13 ± 2.85^{Bl}
3-hydroxy-4-methoxy cinnamic acid	89.40 ± 2.96^{Cb}	30.19 ± 2.65^{Ec}	144.22 ± 2.84^{Aa}	98.70 ± 3.21^{Bl}	145.96 ± 3.84^{Ak}	71.86 ± 4.78^{Dm}
Myricetin	122.64 ± 4.32^{Db}	94.79 ± 2.85^{Ec}	311.82 ± 9.12^{Aa}	310.39 ± 9.85^{Ak}	241.37 ± 5.45^{Bl}	194.10 ± 6.59^{Cm}
Quercetin	2.94 ± 0.56^{Db}	1.13 ± 0.13^{Ec}	4.67 ± 1.12^{Ca}	9.67 ± 1.25^{Bl}	11.35 ± 1.84^{Ak}	4.36 ± 0.19^{Cm}
Kaempferol	8.87 ± 1.12^{Db}	3.82 ± 0.41^{Ec}	21.49 ± 1.45^{Ca}	70.19 ± 7.84^{Ak}	21.42 ± 2.14^{Cm}	28.07 ± 1.18^{Bl}
Rutin hydrate	23.93 ± 1.45^{Eb}	8.53 ± 0.45^{Fc}	83.39 ± 3.12^{Ca}	236.59 ± 6.10^{Ak}	226.05 ± 6.95^{Bl}	41.86 ± 2.14^{Dm}
Naringin	119.57 ± 2.34^{Db}	117.91 ± 2.95^{Db}	288.42 ± 4.59^{Ca}	343.55 ± 6.25^{Ak}	326.60 ± 6.54^{ABkl}	313.44 ± 5.61^{Bl}
Hesperidin	2.69 ± 0.16^{Db}	2.30 ± 0.16^{Db}	3.55 ± 0.48^{Ca}	12.66 ± 1.42^{Ak}	12.27 ± 2.14^{Ak}	8.46 ± 1.26^{Bl}
Neohesperidin	1.23 ± 0.14^{DEb}	0.94 ± 0.05^{Ec}	1.66 ± 0.12^{Da}	3.58 ± 0.95^{Bl}	2.97 ± 0.23^{Cm}	6.14 ± 1.14^{Ak}
(+)-Catechin	1.70 ± 0.19^{Fc}	5.63 ± 0.96^{Eb}	32.39 ± 0.41^{Ba}	7.21 ± 1.58^{Dm}	23.91 ± 1.45^{Cl}	47.75 ± 4.40^{Ak}
(-)-Epicatechin	262.33 ± 2.64^{Db}	61.12 ± 2.46^{Ec}	661.53 ± 9.65^{Ca}	2599.73 ± 29.41^{Bl}	3675.69 ± 42.41^{Ak}	622.78 ± 9.98^{Cm}
(-)-Epigallocatechin	35.34 ± 1.97^{Eb}	28.40 ± 1.85^{Fc}	89.71 ± 5.12^{Da}	195.44 ± 2.52^{Bl}	254.97 ± 6.95^{Ak}	138.53 ± 3.48^{Cm}
Cyanidin-3-*O*-glucoside chloride	2079.27 ± 6.45^{Db}	444.10 ± 3.47^{Ec}	5297.84 ± 23.14^{Ba}	3649.42 ± 10.85^{Cl}	6154.05 ± 58.42^{Ak}	153.29 ± 3.87^{Fm}
Malvidine-3-*O*-glucoside chloride	3891.21 ± 5.23^{Db}	1566.54 ± 5.42^{Ec}	12933.81 ± 45.87^{Aa}	6095.81 ± 19.84^{Cl}	7985.69 ± 23.87^{Bk}	1520.40 ± 27.95^{Em}
Resveratrol	1.07 ± 0.12^{DEab}	0.97 ± 0.04^{Eb}	1.12 ± 0.14^{Da}	1.37 ± 0.10^{Cm}	2.18 ± 0.12^{Bl}	2.58 ± 0.13^{Ak}

* E: Erdek, K. Kapıdağ, DR: Dried, F: Fruit, 1-3: Codes of samples collected from different regions, ** Mean values (mg/kg)±standard deviation (N=3×2) with different capital letters (A-F) in the same row are significantly different (p<0.05) according to collected from different region at dried fruit. Mean values±standard deviation (N=3×2) with different lowercase (a-c. k-m) in the same row are significantly different (p<0.05) according to collected from the same region at dried fruit.

Tab. 3: Phenolic compounds concentrations of fresh *Vac. myrtillus* leaves grown in Erdek ve Kapıdağ regions (mg/kg)

Phenolic compounds	E1FRL*	E2FRL	E3FRL	K1FRL	K2FRL	K3FRL
Gallic acid	12.09 ± 1.65^{Cb**}	33.33 ± 2.14^{Ba}	6.53 ± 0.96^{Ec}	46.64 ± 3.58^{Ak}	12.53 ± 1.26^{Cl}	7.17 ± 1.12^{Dm}
Vanillic acid	159.69 ± 6.85^{Ba}	92.79 ± 6.51^{Cb}	48.28 ± 3.48^{Ec}	18.00 ± 2.14^{Fm}	167.59 ± 15.20^{Ak}	62.69 ± 9.87^{Dl}
Caffeic acid	248.76 ± 9.78^{Aa}	197.65 ± 12.85^{Bb}	28.66 ± 2.14^{Dc}	51.85 ± 4.59^{Ck}	27.82 ± 3.85^{Dl}	14.49 ± 2.15^{Em}
Chlorogenic acid	9.07 ± 1.23^{Aa}	3.16 ± 0.29^{Bc}	3.41 ± 0.27^{Bb}	2.27 ± 0.52^{CDl}	2.20 ± 0.84^{Dl}	2.49 ± 0.48^{Ck}
Syringic acid	31.93 ± 2.14^{Cb}	960.56 ± 15.26^{Aa}	26.05 ± 3.56^{Dc}	24.18 ± 3.65^{El}	130.15 ±10.84^{Bk}	24.09 ± 2.47^{El}
***p*-Coumaric acid**	123.44 ± 6.54^{Aa}	69.26 ± 6.58^{Cb}	50.35 ± 5.84^{Dc}	92.83 ± 9.85^{Bl}	129.87 ± 15.20^{Ak}	36.23 ± 3.65^{Em}
Ferulic acid	5.51 ± 1.10^{BCa}	5.23 ± 0.95^{Db}	5.33 ± 1.10^{CDab}	5.59 ± 1.05^{BCl}	5.68 ± 0.95^{Bl}	6.50 ± 0.98^{Ak}
Trans-ferulic acid	9.98 ± 1.18^{Bb}	11.68 ± 1.84^{Aa}	5.19 ± 0.56^{Cc}	4.48 ± 0.96^{Dl}	4.38 ± 0.28^{Dl}	5.27 ± 0.82^{Ck}
3-hydroxy-4-methoxy cinnamic acid	68.30 ± 3.87^{Ba}	35.45 ± 3.58^{Fc}	59.22 ± 3.98^{Cb}	49.34 ± 4.12^{Dl}	86.71 ± 6.54^{Ak}	41.80 ± 6.14^{Em}
Myricetin	118.02 ± 6.41^{Ba}	57.06 ± 4.50^{Dc}	87.53 ± 6.52^{Cb}	84.26 ± 9.23^{Cl}	141.45 ± 8.59^{Ak}	56.33 ± 10.20^{Dm}
Quercetin	2.26 ± 0.25^{Db}	0.99 ± 0.19^{Ec}	9.32 ± 1.12^{Ca}	9.97 ± 1.10^{BCm}	11.83 ± 1.65^{Ak}	10.84 ± 3.45^{ABl}
Kaempferol	2.56 ± 0.39^{Cb}	1.28 ± 0.25^{Fc}	9.91 ± 1.48^{Aa}	1.79 ± 0.27^{Dl}	1.38 ± 0.29^{Em}	8.78 ± 2.15^{Bk}
Rutin hydrate	5.74 ± 0.48^{Dc}	24.18 ± 1.17^{Aa}	9.81 ± 2.00^{Bb}	5.53 ± 0.52^{Dl}	2.46 ± 0.54^{Em}	8.95 ± 1.84^{Ck}
Naringin	21.66 ± 1.85^{Aa}	4.45 ± 0.98^{Dc}	14.39 ± 2.58^{Bb}	5.10 ± 0.29^{Dl}	13.40 ± 2.87^{Ck}	14.04 ± 2.14^{BCk}
Hesperidin	13.48 ± 1.74^{Aa}	7.50 ± 1.25^{Cc}	9.56 ± 2.05^{Bb}	6.31 ± 0.84^{Dl}	5.89 ± 0.68^{Em}	7.73 ± 0.96^{Ck}
Neohesperidin	2.81 ± 0.69^{BCb}	2.68 ± 0.62^{Cb}	7.73 ± 2.14^{Aa}	2.36 ± 0.26^{Dl}	2.19 ± 0.39^{Em}	3.12 ± 0.25^{Bk}
(+)-Catechin	33.20 ± 2.98^{Ecc}	47.15 ± 3.48^{Cb}	61.71 ± 9.51^{Ba}	78.83 ± 8.63^{Ak}	44.65 ± 5.62^{Dl}	22.51 ± 2.15^{Fm}
(-)-Epicatechin	18.72 ± 2.45^{Ba}	7.06 ± 1.18^{Db}	5.60 ± 1.10^{Ec}	2.55 ± 0.35^{Fm}	14.00 ± 2.10^{Cl}	20.55 ± 2.46^{Ak}
(-)-Epigallocatechin	9.84 ± 2.12^{Cc}	26.79 ± 3.15^{Bb}	61.26 ± 8.56^{Aa}	8.70 ± 0.95^{Dk}	7.23 ± 1.98^{El}	9.12 ± 1.10^{CDk}
Cyanidin-3-*O*-glucoside chloride	1.51 ± 0.23^{Ba}	1.02 ± 0.20^{Cb}	0.96 ± 0.18^{Db}	0.98 ± 0.27^{CDl}	0.97 ± 0.19^{CDl}	23.61 ± 2.58^{Ak}
Malvidine-3-*O*-glucoside chloride	1.57 ± 0.15^{Ba}	1.10 ± 0.14^{Cb}	0.96 ± 0.12^{Db}	1.05 ± 0.16^{CDl}	0.00 ± 0.00^{Em}	42.66 ± 5.00^{Ak}
Resveratrol	1.57 ± 0.10^{Ec}	4.11 ± 0.58^{Cb}	6.29 ± 1.10^{Aa}	3.91 ± 0.56^{Dm}	5.89 ± 0.84^{ABk}	5.31 ± 0.85^{Bl}

[*] E: Erdek, K: Kapıdağ, FR: Fresh, L: Leaf, 1-3: Codes of samples collected from different regions, [**] Mean values (mg/kg)±standard deviation (N=3×2) with different capital letters (A-F) in the same row are significantly different (p<0.05) according to collected from different region at fresh leaf. Mean values±standard deviation (N=3×2) with different lowercase (a-c. k-m) in the same row are significantly different (p<0.05) according to collected from the same region at fresh leaf.

Tab. 4: Phenolic compounds concentrations of dried *Vac. myrtillus* leaves grown in Erdek ve Kapıdağ regions (mg/kg)

Phenolic compounds	E1DRL*	E2DRL	E3DRL	K1DRL	K2DRL	K3DRL
Gallic acid	176.95 ± 6.98^{Cc**}	352.30 ± 3.59^{Aa}	201.37 ± 3.29^{Bb}	203.48 ± 4.36^{Bk}	67.43 ± 3.69^{Em}	110.28 ± 9.62^{Dl}
Vanillic acid	271.17 ± 5.89^{Bb}	1156.80 ± 18.29^{Aa}	249.72 ± 4.62^{Cc}	157.27 ± 2.48^{Dl}	230.63 ± 13.52^{Ck}	20.78 ± 2.98^{Em}
Caffeic acid	27.05 ± 3.21^{Aa}	5.28 ± 1.14^{Cb}	28.24 ± 2.12^{Aa}	8.61 ± 1.10^{Bk}	4.65 ± 0.85^{Dl}	3.28 ± 0.68^{Em}
Chlorogenic acid	3.31 ± 0.45^{Ba}	3.23 ± 0.58^{Ca}	1.25 ± 0.26^{Eb}	4.14 ± 0.87^{Ak}	1.94 ± 0.34^{Dm}	3.25 ± 0.52^{BCl}
Syringic acid	202.99 ± 5.47^{Aa}	84.36 ± 6.47^{Dc}	113.56 ± 3.29^{Bb}	95.71 ± 6.54^{Ck}	47.56 ± 3.85^{El}	32.46 ± 5.20^{Fm}
***p*-Coumaric acid**	15.93 ± 2.85^{Ba}	11.71 ± 2.19^{Dc}	13.18 ± 1.23^{Cb}	8.51 ± 1.98^{Em}	15.24 ± 2.18^{Bl}	17.58 ± 2.16^{Ak}
Ferulic acid	3.13 ± 0.95^{Eb}	3.93 ± 0.48^{Ca}	3.80 ± 0.48^{CDa}	9.99 ± 2.57^{Ak}	3.50 ± 0.84^{DEm}	6.56 ± 1.05^{Bl}
Trans-ferulic acid	12.29 ± 2.14^{Db}	11.90 ± 2.43^{DEb}	24.93 ± 1.15^{Aa}	16.03 ± 2.18^{Bk}	15.89 ± 2.17^{Cl}	10.95 ± 2.84^{Em}
3-hydroxy-4-methoxy cinnamic acid	111.93 ± 4.58^{Cb}	254.11 ± 4.56^{Aa}	88.46 ± 3.58^{Ec}	141.86 ± 9.84^{Bk}	96.34 ± 9.27^{Dl}	51.75 ± 9.13^{Fm}
Myricetin	101.45 ± 4.32^{Db}	237.56 ± 5.42^{Aa}	94.76 ± 4.51^{Dc}	152.97 ± 11.18^{Bk}	140.59 ±12.17^{Cl}	49.44 ± 7.68^{Em}
Quercetin	4.63 ± 0.47^{Cb}	2.07 ± 0.23^{Dc}	8.10 ± 1.18^{Ba}	4.27 ± 0.87^{Cl}	2.75 ± 0.96^{Dm}	11.38 ± 2.34^{Ak}
Kaempferol	1.63 ± 0.28^{Dc}	3.37 ± 0.43^{Aa}	2.17 ± 0.18^{Bb}	2.20 ± 0.59^{Bl}	3.30 ± 0.95^{Ak}	2.07 ± 0.56^{Bl}
Rutin hydrate	12.27 ± 1.15^{Dc}	79.69 ± 6.58^{Aa}	16.63 ± 2.15^{Cb}	21.79 ± 2.51^{Bk}	11.98 ± 3.25^{El}	8.08 ± 1.02^{Fm}
Naringin	95.12 ± 5.21^{Bb}	261.38 ± 5.53^{Aa}	83.45 ± 6.41^{Cc}	34.66 ± 3.26^{El}	43.08 ± 8.26^{Dk}	1.14 ± 0.38^{Fm}
Hesperidin	82.06 ± 5.10^{Bb}	134.01 ± 4.87^{Aa}	60.14 ± 4.28^{Cc}	36.93 ± 3.48^{Dk}	7.45 ± 2.14^{Fm}	9.74 ± 1.36^{El}
Neohesperidin	6.52 ± 0.75^{Ba}	3.18 ± 0.59^{Cb}	6.06 ± 0.58^{Ba}	17.24± 2.47^{Ak}	2.63 ± 0.51^{Cm}	16.20 ± 2.01^{Al}
(+)-Catechin	17.50 ± 1.48^{Db}	95.59 ± 3.57^{Aa}	21.94 ± 2.43^{Bb}	19.73 ± 2.52^{Cl}	7.31 ± 2.18^{Em}	22.71 ± 3.20^{Bk}
(-)-Epicatechin	49.08 ± 3.47^{Ba}	12.78 ± 2.15^{Db}	10.06 ± 1.64^{Dc}	84.06 ± 9.52^{Ak}	37.31 ± 6.20^{Cm}	49.38 ± 4.02^{Bl}
(-)-Epigallocatechin	52.07 ± 3.65^{Ca}	18.20 ± 2.39^{Ec}	28.78 ± 3.28^{Db}	67.26 ± 6.53^{Bl}	29.84 ± 3.27^{Dm}	197.80 ± 12.10^{Ak}
Cyanidin-3-*O*-glucoside chloride	0.00 ± 0.00^{Cb}	1.00 ± 0.06^{ABa}	1.01 ± 0.17^{ABa}	1.06 ± 0.26^{Ak}	1.01 ± 0.18^{ABkl}	0.95 ± 0.09^{Bl}
Malvidine-3-*O*-glucoside chloride	1.09 ± 0.18^{ABa}	0.00 ± 0.00^{Cb}	1.14 ± 0.16^{ABa}	1.20 ± 0.19^{Ak}	1.11 ± 0.24^{ABkl}	0.95 ± 0.12^{Bl}
Resveratrol	3.32 ± 0.42^{Ba}	1.54 ± 0.28^{Cb}	1.50 ± 0.45^{Cb}	8.89 ± 2.21^{Ak}	8.55 ± 1.00^{Ak}	3.85 ± 0.45^{Bl}

[*] E: Erdek, K. Kapıdağ, DR: Dried. L: Leaf, 1-3: Codes of samples collected from different regions, [**] Mean values (mg/kg)±standard deviation (N=3×2) with different capital letters (A-F) in the same row are significantly different (p<0.05) according to collected from different region at dried leaf. Mean values±standard deviation (N=3×2) with different lowercase (a-c. k-m) in the same row are significantly different (p<0.05) according to collected from the same region at dried leaf.

chin, and (-)-epigallocatechin compared to fresh fruit and leaf and also dried leaf extracts. The flavanol contents in fruit and leaf extracts were determined to be increasing or decreasing depending on the altitude, the area, and the harvesting time where the samples were collected as well as the drying process. Drying and the drying conditions (oxygen, high temperature, length of time, without vacuum, etc.) may cause polymerization reactions, reduce some polyphenols and their antioxidant capacity, and induce the formation of new compounds (KENDARI et al., 2012). As a stated by KENDARI et al. (2012) catechin oxidation mechanism initially constructs a semiquinon, which then converts into a quinon. The quinon compound can react with amino acid, protein, or other polymers to produce proanthocyanidin. And then proanthocyanidin can degrade into catechin because proanthocyanidin represents an oligomer or polymer from flavan-3-ol (catechin/epicatechin). These flavanols have already been detected in some berry fruit and leaf (SELLAPPAN et al., 2002; HARRIS et al., 2007; VRHOVSEK et al., 2012; DENG et al., 2014).

In blueberry skin and flesh, delphinidin, cyanidin, petunidin, peonidin, and malvidin monoglycosides are the main anthocyanins. Because of the diversity in the glycosylation and acylation pattern, more than 25 anthocyanins have been identified in blueberries (HARRIS et al., 2007; MOZE et al., 2011). The amounts and distribution of anthocyanins in the berries differ depending on their plant species, cultivation conditions in which the fruit have been grown or stored (e.g. light, temperature), and producing districts, due to genetic differences among wild and cultivated varieties (EHLENFELDT and PRIOR, 2001). Blueberry leaves are by-products of the blueberry industry. The leaves contain in an excessively amount of polyphenols, which creates an opportunity for their use in the neutraceutical industry, more so than the fruits (EHLENFELDT and PRIOR, 2001; KIM and UM, 2011; LI et al., 2013; DENG et al., 2014). But no anthocyanins have been found in fresh green leaves (HARRIS et al., 2007; LI et al., 2013). We found fresh and dried *Vac. myrtillus* fruit extracts with high levels of malvidin-3-*O*-glucoside chloride (212.93-4433.19 mg/kg FW and 1520.40-12933.81 mg/kg DW) and cyanidin-3-*O*-glucoside chloride (70.94-781.26 mg/kg FW and 153.29-6154.05 mg/kg DW) collected from Turkey, respectively. As mentioned earlier, fresh and dried blueberry leaf extracts have significantly less ($p<0.05$) anthocyanins than fruit extracts. Nevertheless, there were numerous reports that anthocyanins were detected in blueberry leaves (HARRIS et al., 2007; KIM and UM, 2011). These differences are possibly related to the harvesting season of leaves. Within the anthocyanidin reductase or leucoanthocyanidin reductase activity, the transcriptional control favors the expression of the anthocyanidin synthase in sun-exposed leaves. The decrease in proanthocyanidin content in favor of anthocyanin production has also been observed during fruit development at the point when leaves turned red, activating the biosynthesis of cyanidin glycosides (JAAKOLA et al., 2004; LI et al., 2013). In a previous study reported by LI et al. (2013) and RIIHINEN et al. (2008) anthocyanins were not detected in green leaves of blueberry and bilberry, but a low amount was detected in red leaves. Resveratrol is a type of natural phenol and a phytoalexin produced naturally by several plants in response to injury or when the plant is under attack by pathogens such as bacteria or fungi (FRÉMONT, 2000). The occurrence of resveratrol in *Vaccinium* berries should not be surprising, as its occurrence in the plant kingdom appears to be widespread (RIMANDO et al., 2004). Food sources of resveratrol include the skin of grapes, blueberries, raspberries, and mulberries (JASIŃSKI et al., 2013) and the variability in the resveratrol content in fruit such as blueberries changes due to food processing or preparation. Recently, resveratrol was reported by LYONS et al. (2003) at levels of approximately 0.0002-0.0006 ng/g sample (RIMANDO et al., 2004). The resveratrol contents of blueberries and the related bilberry, *Vac. myrtillus* L., were cultivated in several different geographical regions (LYONS et al., 2003). In our study, resveratrol was found in fresh fruit and leaf extracts (0.70-1.01 mg/kg FW and 1.57-6.29 mg/kg FW, respectively) from both regions. The previous data for trans-resveratrol content (0.4 mg/100 g FW) in blueberry (MOZE et al., 2011) agreed with ours, but it has also been determined to be lower for blueberries (WANG et al., 2008). Also, as stated by LYONS et al. (2003), blueberries and bilberries were found to contain resveratrol and the level of this chemoprotective compound in these fruits was <10% of that reported for grapes. In grapes, the levels of resveratrol were found to vary with the time of harvest, environmental and climatic conditions,

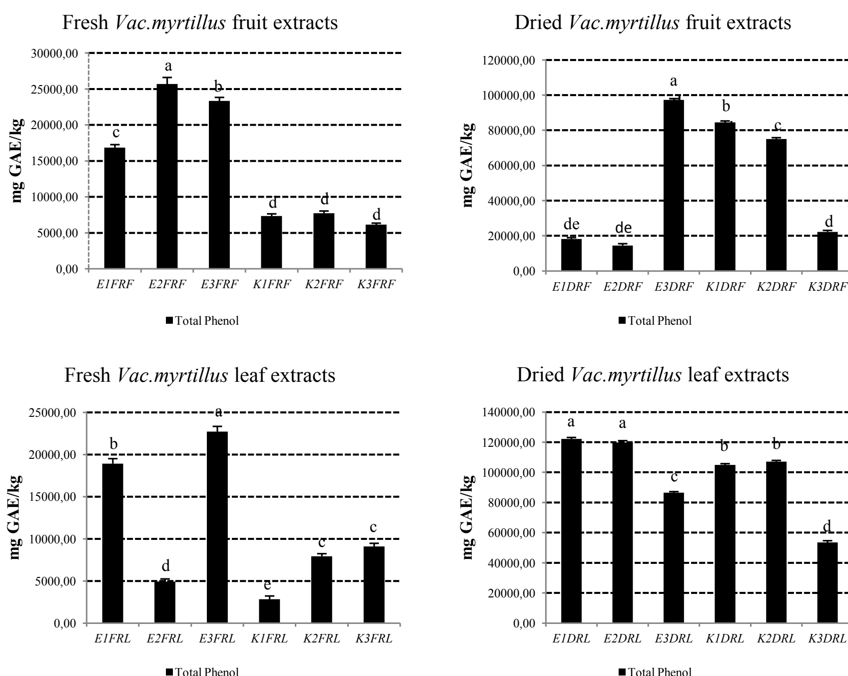

Fig. 1: Total phenolic (TP) contents (mg GAE/kg) of fresh and dried *Vac. myrtillus* fruit and leaf extracts (*E*: Erdek, *K*. Kapıdağ, *FR*: Fresh, *DR*: Dried, *F*: Fruit, *L*: Leaf, 1-3: Codes of samples collected from different regions, Different letter(s) on bar indicate statistically significant differences, $p<0.05$)

and plant developmental stage (RIMANDO et al., 2004). These factors possibility affected the differences in resveratrol contents of the *Vac. myrtillus* extracts in this study.

Total phenol and total anthocyanin contents

The TP contents were significantly different among the fruit and leaf *Vac. myrtillus* extracts (Fig. 1). The TP was 6152.05-25688.90 mg GAE/kg in fresh fruit extracts, whereas in dried fruit were determined to be 14512.49-97214.25 mg GAE/kg. For the fresh and dried blueberry leaf extracts, their TP contents were significantly ($p<0.05$) higher than those in fresh and dried fruits. In the leaf tissues of 87 highbush blueberries, the mean values of polyphenol contents were ~30 times higher than observed in fruits on a FW basis (EHLENFELDT and PRIOR, 2001). SKUPIEŃ et al. (2006) reported that the TP contents in *Vac. corymbosum* L. leaf extracts was 111.5 mg/100 g dw dried leaves. In addition, the TP of the leaf extracts of *Vac. myrtillus* in Erdek and Kapıdağ regions were significatly higher ($p<0.05$) than those of blackberry leaves (82.8-91.6 mg of GAE/g of DW) and strawberry leaves (55.2 mg of GAE/g of DW) reported by WANG and LIN (2000). LI et al. (2013) and OSZMIAŃSKI et al. (2011) indicated that the polyphenol content of the blueberry leaves was much higher than those of any other leaves of tested berries (blackberry, raspberry, honeyberry, and strawberry). The differences in TP contents between fruit and leaf extracts were statistically significant ($p<0.05$). Generally, the TP of plant extracts can be affected by solvent, its polarity, its concentration, and/or extraction method method (DENG et al., 2014; XIAOYONG and LUMING, 2014). It is also well known that genetic, agronomic or environmental factors play important roles in phenolic composition and nutritional quality of crops (YANG et al., 2009). When the TP contents of these extracts are compared with white wines, these plants could contribute the same health benefit as those wines in terms of polyphenols. A large variation was observed among fruit and leaf extracts for TA content (Fig. 2), ranging from 2805.08 to 5973.69 mg/kg (in fresh fruit), from 2094.56 to 5975.14 mg/kg (in dried fruit), from 12.89 to 262.02 mg/kg (in fresh leaf), and from 51.91 to 318.30 mg/kg (in dried leaf). Fresh and dried fruit samples are a good source of anthocyanin (Tab. 1-4), however, fresh fruit samples recorded decreases/increases in TA content. The TA content in blueberry fruit and leaf extracts in this study was comparable to the quantity reported by EHLENFELDT and PRIOR (2001), SELLAPPAN et al. (2002), LOHACHOOMPOL et al. (2008), and WANG et al. (2015). Results obtained from several studies suggest that the TP and TA contents in blueberry fruit are influenced by the cultivar, harvest time, the growing season, fruit mass, maturity, environmental growing conditions, growing location, postharvest storage conditions, drying process, different extraction methods, irridation, temperature, and pathogen attacks (ROUTRAY et al., 2014).

Antioxidant capacity

Blueberry fruit and leaf show high antioxidant capacity, correlated especially with their anthocyanin and other phenolic compounds content, and may be considered as one of the highest antioxidant sources among fruits and vegetables (VRHOVSEK et al., 2012). The values found in the fresh *Vac. myrtillus* fruit extracts for antioxidant capacity (Fig. 3) were 8.37-23.26 µmol TE/g FW by CUPRAC, 8.56-19.23 µmol TE/g FW by DPPH, 4.26-9.56 µmol TE/g FW by ABTS, and 0.97-1.73 µmol TE/g FW by FRAP method, values lower/higher than or close to those found in fresh leaf extracts (Fig. 4). Also in blueberry fruits and leaves studied by WANG and LIN (2000), EHLENFELDT and PRIOR (2001), SELLAPPAN et al. (2002), LI et al. (2013), and WANG et al. (2015) using the same methods, the *Vac. myrtillus* fruit and leaf extract results obtained in this study were similar to those studies. Therefore, as a reported by WANG and LIN (2000) and PARK et al. (2012), because of their high antioxidant content, blueberry leaves can also be added to tea mixes to increase the antioxidant capacity level in the beverages for greater benefits to human health.
Previous studies found a direct relationship between the antioxidant capacity, and the TP and TAs contents in blueberry fruits and leaves (EHLENFELDT and PRIOR, 2001; LI et al., 2013). In this study, there was a strong correlation between antioxidant capacity and TP content

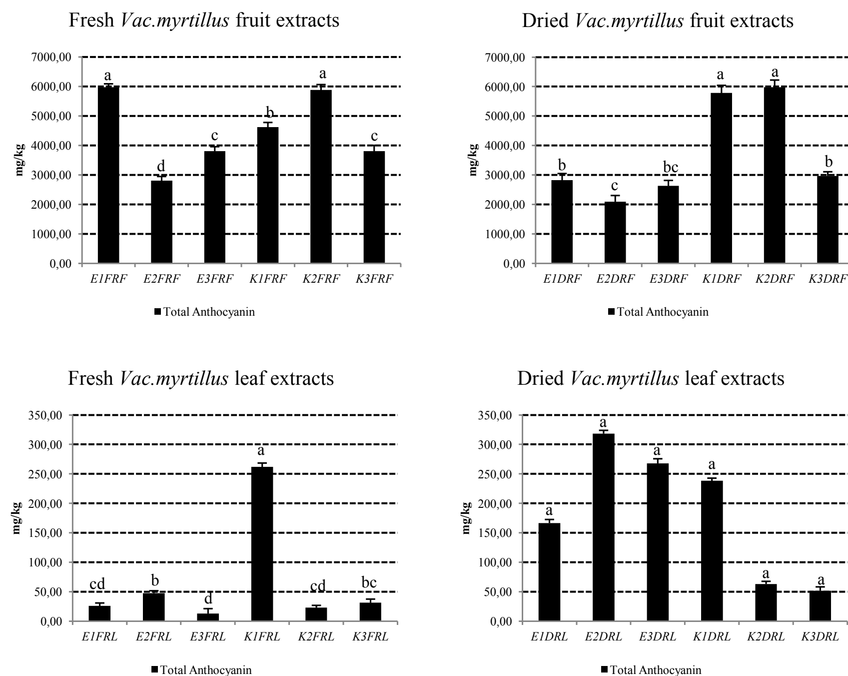

Fig. 2: Total anthocyanin (TA) contents (mg/kg) of fresh and dried *Vac. myrtillus* fruit and leaf extracts (*E*: Erdek, *K*. Kapıdağ, *FR*: Fresh, *DR*: Dried, *F*: Fruit, *L*: Leaf, 1-3: Codes of samples collected from different regions, Total anthocyanins were expressed as cyanidin-3-glucoside equivalents, Different letter(s) on bar indicate statistically significant differences, $p<0.05$)

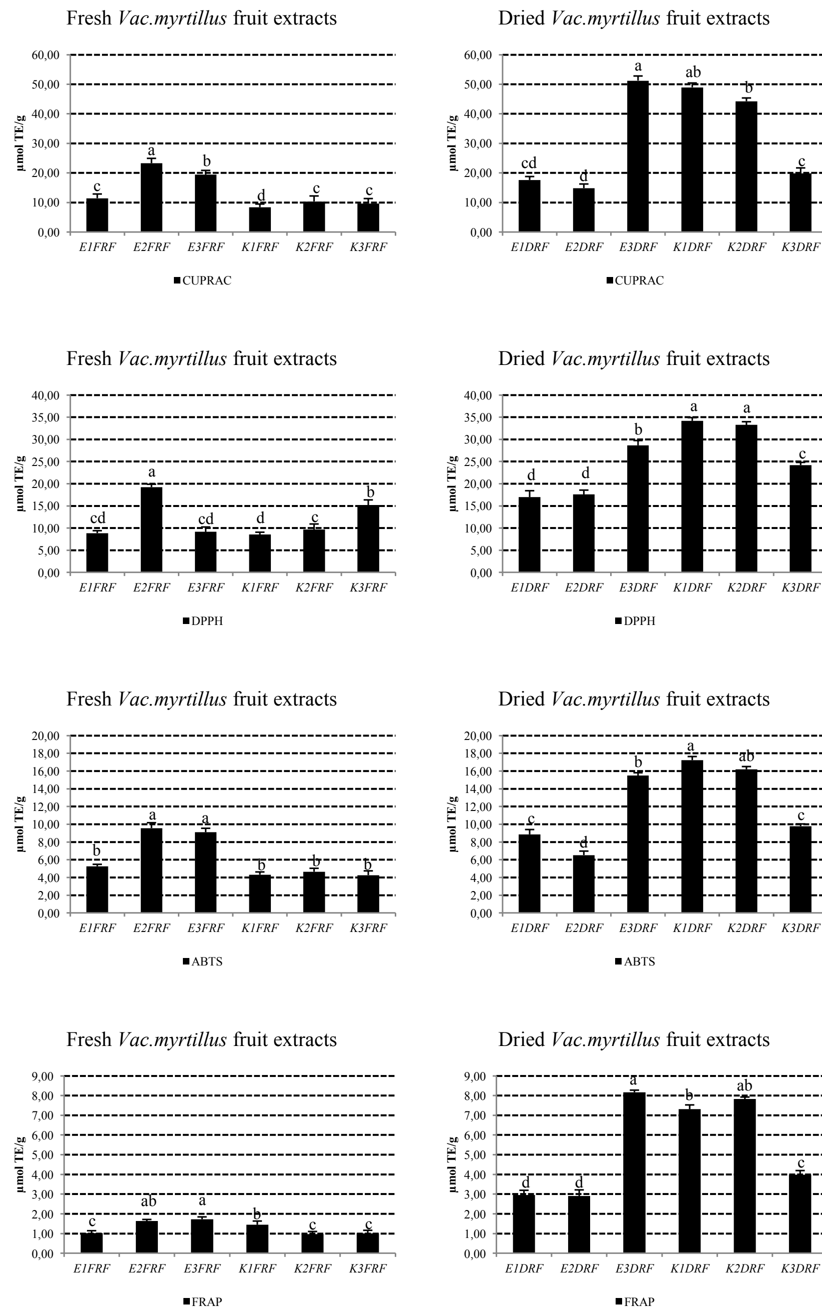

Fig. 3: Antioxidant capacities (µmol TE/g) of fresh and dried *Vac. myrtillus* fruit extracts (E: Erdek, K. Kapıdağ, FR: Fresh, DR: Dried, F: Fruit, 1-3: Codes of samples collected from different regions, Different letter(s) on bar indicate statistically significant differences, $p<0.05$)

in the dried fruit and fresh leaf extracts, while weak correlation TA content in fresh fruit extracts (Tab. 5, is available in Supplementary material). These results indicated that the phenolics, rather than the anthocyanins alone, play an important role in contributing to the whole antioxidant capacity. The reason may be that anthocyanins in fruit and leaves could transform into other types of phenolics, which have higher levels of antioxidant capacity, via the drying and sample preparation processes. As a reported by previously study, in the dry heating experiment, all phenolics including anthocyanins in the blueberry pomace were completely degraded to small fragments that had no antioxidant capacity. Thus, the loss of the anthocyanins and other phenolics straightforwardly linked to the decrease of antioxidant capacity of the dry-heated pomace (Bener et al., 2013).

Conclusion

The drying method affects the quality of the end products such as color, texture, aroma, along with its chemical constituents (Routray et al., 2014). Therefore, the control method chosen during this study was fresh storage after vacuum packaging. Oven-dried fruit and leaf samples retained higher/lower amounts of phenolic content, TP and TA according to the degree of resistance to the drying process of the phenolic compounds and the growing season, suggesting that enviromental growing conditions, harvesting altitude, and length and type of drying time used for the dried samples when compared to the amount obtained from fresh samples. Also, anthocyanins, flavonols, and proanthocyanidins are located mainly in the peel while hydroxycinnamates are found in the flesh (Golding et al., 2001), therefore, it is believe that they are affected by different degrees

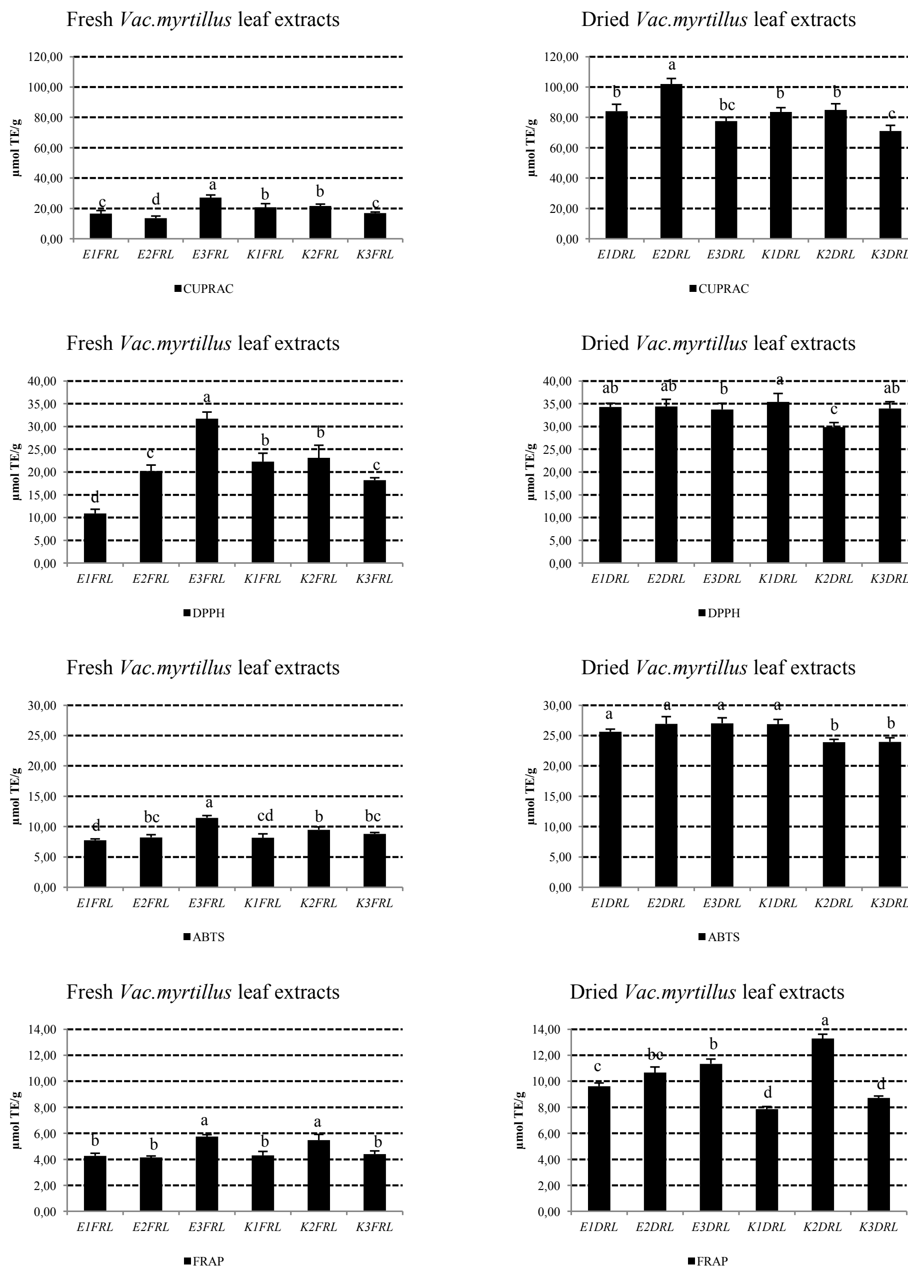

Fig. 4: Antioxidant capacities (µmol TE/g) of fresh and dried *Vac. myrtillus* leaf extracts (E: Erdek, K. Kapıdağ, FR: Fresh, DR: Dried, L: Leaf, 1-3: Codes of samples collected from different regions, Different letter(s) on bar indicate statistically significant differences, $p<0.05$)

from an applied drying method, while the antioxidant capacities of the samples increase under the same conditions. If combined with the other protective methods, oven-drying proved to be a suitable method for *Vac. myrtillus* samples preservation because the phenolic compounds and their functional properties were either increased or at least decreased. Consequently, *Vac.myrtillus* fruit and leaf can be recommended as an addition to food composition, to increase the antioxidant capacity, because of their high antioxidant properties.

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Supplementary material

Tab. 5: Correlation coefficient of total phenolic content. total anthocyanin content and antioxidant capacity of fresh and dried *Vac. myrtillus* fruit and leaf extracts

Fresh fruit extract	Total phenol	Total anthocyanin	CUPRAC	DPPH	ABTS	FRAP
Total phenol	1	-0.406	0.935**	0.323	0.984**	0.704
Total anthocyanin	-0.406	1	-0.679	-0.774	-0.638	-0.632
CUPRAC	0.935**	-0.679	1	0.545	0.987**	0.745
DPPH	0.323	-0.774	0.545	1	0.420	0.187
ABTS	0.948**	-0.638	0.987**	0.420	1	0.811
FRAP	0.704	-0.632	0.745	0.187	0.811	1

Dried fruit extract	Total phenol	Total anthocyanin	CUPRAC	DPPH	ABTS	FRAP
Total phenol	1	0.572	0.997**	0.869*	0.949**	0.981**
Total anthocyanin	0.572	1	0.630	0.835*	0.777	0.642
CUPRAC	0.997**	0.630	1	0.900*	0.970**	0.984**
DPPH	0.869*	0.835*	0.900*	1	0.955**	0.919**
ABTS	0.949**	0.777	0.970**	0.955**	1	0.960**
FRAP	0.981**	0.642	0.984**	0.919**	0.960**	1

Fresh leaf extract	Total phenol	Total anthocyanin	CUPRAC	DPPH	ABTS	FRAP
Total phenol	1	0.861*	0.981**	0.738	0.957**	0.918**
Total anthocyanin	0.861*	1	0.932**	0.659	0.925**	0.930**
CUPRAC	0.981**	0.932**	1	0.782	0.978**	0.958**
DPPH	0.738	0.659	0.782	1	0.814*	0.814*
ABTS	0.957**	0.925**	0.978**	0.814*	1	0.992**
FRAP	0.918**	0.930**	0.958**	0.814*	0.992**	1

Dried leaf extract	Total phenol	Total anthocyanin	CUPRAC	DPPH	ABTS	FRAP
Total phenol	1	0.474	0.793	-0.020	0.420	0.238
Total anthocyanin	0.474	1	0.607	0.576	0.971**	-0.131
CUPRAC	0.793	0.607	1	0.048	0.444	0.231
DPPH	-0.020	0.576	0.048	1	0.653	-0.863*
ABTS	0.420	0.971**	0.444	0.653	1	-0.250
FRAP	0.238	-0.131	0.231	-0.863*	-0.250	1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).