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Growing conditions effect on fruit phytochemical composition and anti-microbial activity of plum (cv. Black Diamond)

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Abstract

Meteorological parameters such as temperature, atmospheric pressure, humidity, radiation, precipitation, wind speed etc. vary between the regions where are located at the same latitude with different altitudes. High correlation between plant characteristics and climatic parameters leads researchers to reveal how plants react to different environmental conditions. In this study, some phytochemical and antimicrobial characteristics fruits of 'Black Diamond' plum variety which were harvested at two different altitudes (200 m and 800 m), during two consecutive years (2017 and 2018) were investigated fort the explain of altitude change effects on these characteristics. As a result of the study, it was determined that the amount of phenolic compounds and organic acids were increased with altitude increase while antimicrobial activities were decreased. Ranking of organic acids in both altitudes was found as malic acid> acetic acid> oxalic acid> ascorbic acid. In terms of phenolic acids, a stable order could not be determined. Acetic acid and chlorogenic acid were found as a strong anti-bacterial compounds. Same results obtain for oxalic acid, malic acid, ascorbic acid, routine, parahydroxybenzoic acid, p-coumaric acid, gallic acid, protocatechuic acid, vanilic acid, caffeic acid and syringic acid in terms of anti-fungal activity.

Key words: Phenolic compounds, organic acids, anti-microbial activity, altitude

Introduction

The most important factor affecting the productivity of agricultural activities is the suitability of ecological factors. In regions with different climatic features, changes occur in the morphological and biochemical characteristics of fruits belonging to the same variety (Bugaud et al., 2007; Mikulic-Petkovsek et al., 2015; Usanmaz et al., 2018). The differences in some properties such as latitude, proximity to the water body, topography, and altitude cause variation in climatic features between the regions (Korner, 2007; Bais et al., 2018). Altitude is the vertical distance of any place to the sea level. Ignoring the effects of other factors affecting the climate, it has been observed that there are substantial changes in climatic characteristics related to the altitude change. In general, with the increase in altitude, atmospheric layer thickness, the angle of the sun rays, light intensity, radiation, precipitation, and wind activities increase whereas atmospheric temperature and atmospheric pressure decrease (Korner, 2007; Muniz et al., 2018). In line with this change in meteorological parameters, it has been reported that the phytochemical composition has substantially undergone a change in many fruit species including plums (Guerra and Casquero, 2009; Mikulic-Petkovsek et al., 2015; Palmieri et al., 2017; Lu et al., 2017; Gunduz and Ozbay, 2018).

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Phenol and organic acids commonly found in plum fruits are compounds that show high antioxidant activity by preventing the start of oxidation and peroxidation reactions. This antioxidative effect reduces the risk of many chronic diseases including cancer and cardiovascular diseases (Tang ve Tsao, 2017; Pham et al., 2019). In addition, these compounds are effective against many yeast and bacterial strains that are disease factor. Heritability of phytochemical properties is low and sensitivity to environmental conditions is high. Superior phytochemical properties are possible only cultivation with right variety and suitable ecology. For this reason, researches on the identification and breeding of varieties suitable for ecology have increased recently (Mditshwa et al., 2013; Mezzetti et al., 2016; Blando et al., 2019).

In this study, how the climate change that occurred due to altitude change affects phytochemical and antimicrobial properties in fruits of 'Black Diamond' plum variety was investigated. As a result of the correlation analysis, the antimicrobial potentials of the investigated phytochemical characteristics were determined.

Materials and Methods

The study was carried out in 2017 and 2018 in two different experimental fields which are have different altitudes belonging to the Faculty of Agriculture of Eskisehir Osmangazi University in Turkey. 'Black Diamond', plum cultivars was used as the material. Cultivar was planted in 2011 after grafting onto seedling rootstocks. 40 days after full blooming, thinning was performed on all trees in order to balance the crop load. The fruits were harvested at consumption maturity accordingto the sense of taste, colour and abscission layer (Arion et al., 2014). The fruit of each tree were harvested without mixing with the fruit from other trees and divided into three parallel and each parallel consists of 20 fruit (in total: 3 trees, 9 parallels, and 180 fruits in each region). Then squeezed with a juice extractor to obtain their juices. These juices were used for the phytochemical analyses.

One of the parcels covering the study material is located in Eskişehir-Central region in where typical continental climate is seen, while the other is located in Eskişehir-Sarıcakaya region which is close to the Mediterranean climate and has micro-climate characteristics. Climatic data of the study areas are given in Table 1. While temperature and pressure were higher in Sarıcakaya region, wind speed was higher in Center in all months of both years. Humidity and precipitation were higher in Sarıcakaya, where was warmed earlier in the first months of the years, while higher values were found in the Center in the following months.

Table 1. Average weather data in related months of experimental areas between 2017 –2018 years

	March	April	May	June	July	August	September	October
High Altitude (800 m)								
2017								
Wind speed (m.san^{-1})	3.4	3.0	3.1	3.0	3.6	3.7	2.9	2.6
Temperature (°C)	7.6	9.6	14.4	19.1	23.1	22.0	19.6	10.8
Actual pressure (hPa)	923.2	924.9	923.1	923.9	922.3	923.0	924.7	926.6
Humidity (%)	68.7	66.9	73.2	73.4	59.5	67.3	57.0	72.9
Precipitation (mm.m^{-2})	16.2	62.0	50.8	44.8	13.4	31.4	2.6	46.4
2018								
Wind speed (m.san^{-1})	3.5	2.9	2.9	2.9	3.4	3.1	3.1	2.6
Temperature (°C)	9.2	13.8	16.8	19.9	22.3	22.9	18.6	13.3
Actual pressure (hPa)	920.0	924.9	922.4	921.1	920.8	922.5	925.9	927.9
Humidity (%)	73.5	61.6	74.8	69.5	65.5	63.5	65.4	77.4
Precipitation (mm.m^{-2})	53.6	12.6	62.2	46.6	39.2	18.0	2.8	29.2
Low altitude (200 m)								
2017								
Wind speed (m.san^{-1})	1.9	1.8	1.9	2.1	2.4	2.7	2.1	1.6
Temperature (°C)	11.0	14.2	19.1	23.6	26.7	26.8	24.9	15.3
Actual pressure (hPa)	983.1	984.4	981.4	981.2	978.7	979.5	981.0	985.3
Humidity (%)	67.2	64.5	94.7	77.0	49.9	64.9	62.9	75.5
Precipitation (mm.m^{-2})	32.1	58.4	90.4	48.9	0.8	49.4	2.0	39.4
2018								
Wind speed (m.san^{-1})	1.8	2.1	2.0	2.0	2.4	2.4	2.1	1.7
Temperature (°C)	13.3	18.4	21.4	23.6	26.0	26.7	22.1	16.9
Actual pressure (hPa)	978.7	982.9	979.7	977.6	976.7	978.7	983.2	986.3
Humidity (%)	77.3	70.6	76.0	64.9	50.9	50.4	54.2	66.8
Precipitation (mm.m^{-2})	73.2	14.9	113.3	75.8	37.1	6.2	13.4	44.0

Determination of general phytochemical characteristics

First, juices were centrifuged and the upper part of the solutions were used directly for phytochemical analyses. Total polyphenol content was determined using the colorimetric reaction with the Folin-Ciocalteu reagent (Selcuk and Erkan, 2016). Gallic acid was used as an external standard for the calibration curve and the results were expressed in terms of mg of gallic acid equivalents (mg GAE L^{-1}) of fruit juice. Antioxidant activity analyzes were performed using DPPH method. For this purpose, first of all, fruit juices were mixed and 50% inhibition sample concentration (IC50) was calculated by drawing percent inhibition against the sample concentrations. Then, up to IC 50 value samples were taken and ability to remove the DPPH radical was determined according to the method specified by Polat et al. (2018). Results were expressed as a percentage (%).

Determination of polyphenols and organic acids by HPLC

The polyphenols were separated with an Agilent 1260 series HPLC system equipped with Ace C18 (4.6 mm × 150 mm, 5 μm) column. The flow rate of the mobile phase was kept at 0.5 mL min^{-1} Mobile phase A was water containing 0.02% TFA, and phase B was methanol containing 0.02% TFA. The gradient conditions were as follows: 0-5 min, 25% B; 5-10 min, 25-30% B; 10-16 min, 30-45% B; 16-18 min, 45% B; 18-25 min, 45-80% B; 25-30 min, 80% B; 30-40 min, 80-25% B. The temperature of the column was controlled at 25°C. Injection volume was 10 μL. The detection wavelengths of DAD were set at four positions: 254, 275, 305, and 320 nm (Dawei et al., 2005).

Organic acids were separated with an Agilent 1260 series HPLC system equipped with Ace C18 (4.6 mm × 150 mm, 5 μm) column. Chromatographic separation was performed at 35 °C. The flow rate of the mobile phase was kept at 0.6 mL min^{-1} 0.01 M ammonium hydrogen phosphate $(NH_4)_2HPO_4$ was prepared as mobile phase and pH of the mobile phase was adjusted with H_3PO_4 to 2.4. Injection volume was 10 μL. Ultraviolet Detection (UV) at λ = 210 nm were used for all acids (Fu et al., 2015).

Microorganisms, microbial cultures and antimicrobial activities

Five common microorganisms were used to assess the anti-microbial activity including the Gram-positive *Staphylococcus aureus* (ATCC 29213), Gram-negative *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC 14053), *Candida parapisilozis* (ATCC 22019) yeasts. The well-diffusion method was used to detect the antimicrobial activities of the extracts. Equidistant holes were made in the agar using sterile cork borers (No.9, Ø 11 mm). 100 μL of plum extract was added to the holes using a pipettor. Dishes injected with yeasts were incubated at 25 °C for 48 h and bacterias were incubated at 37 °C for 24 h (İlkimen and Gülbandılar, 2018). At the end of the period, inhibition zones formed on the Müller Hilton Agar were measured as mm. Studies were performed in triplicate, and the developing inhibition zones were compared with those of reference antibiotics (Vancomycin, Cefepime, and Levoflaxacin) and fungicide (Fluconazole).

Statistical analysis

Study was designed in accordance with the completely randomize experimental design with three replicates. The presence of statistical differences in the investigated properties of 'Black Diamond' plum variety were investigated using the t-test procedure in the Minitab-17 package program and pearson correlation coefficients were used to determine the relationships between the characteristics (Düzgüneş et al., 1987).

Results and Discussion The findings of the characteristics examined in the fruit of the 'Black Diamond' variety harvested from different altitudes are given in Table 2. Except for protocatechuic acid, the differences caused by altitude were found to be statistically significant in all of the investigated properties.

Malic acid was determined as the dominant organic acid of different plum varieties by Melgarejo et al. (2012) and Ionika et al. (2013) like the result of our study and the ranking of organic acids at both altitudes was found as malic acid> acetic acid> oxalic acid> ascorbic acid. Amount of oxalic, malic and ascorbic acids were found higher in high altitude while acetic acid was detected higher in low altitude (Table 2).

Although with the increase in altitude, an increase generally has been reported in phenolic compounds and total phenol content (Palmieri et al., 2017; Gunduz and Ozbay, 2018), there are also studies that reported a decrease in these characteristics (Mphahlele et al., 2014). The reaction of plant genotypes to meteorological parameter (radiation, temperature, light, humidity etc.) differences caused by altitude change are different from each other (Mikulic-Petkovsek et al., 2015; Mertoğlu and Evrenosoğlu, 2017). For 'Black Diamond' variety, it was found that total phenol content was increased from 339.8 mg L^{-1} to 817.8 mg L^{-} with altitude increase. Increase in the amount of phenolic compounds may have caused this situation. As a matter of fact, all of the phenolic acids, with the exception of chlorogenic acid, increased in parallel with the altitude increase.

The pH value was measured 3.67 at low altitude while it was decreased to 3.39 in high altitude. Increasing of organic and phenolic acids which are have acidic characteristics at high altitude caused pH to decrease. High degree of negative correlation between organic and phenolic acids with pH were reported in many fruit species such as apple (Vieira et al., 2009; Mertoğlu and Evrenosoğlu, 2019), apricot (Ayour et al., 2017), pomegranate (Fawole ve Opara, 2013), pear (Mertoğlu and Evrenosoğlu, 2019) and including plum (Kitzberger et al., 2017).

Organic and phenolic acids are phytochemicals with high antioxidant properties. These compounds, which increased with the increase in altitude, increased the amount of antioxidant activity in the 'Black Diamond' cultivar from 32.3% to 74.5%. In addition, the increasing amount of UV in parallel with the rise of altitude, increases antioxidant activity by stimulating enzymatic and non-enzymatic defense systems in plants (Martínez-Lüscher et al., 2013).

Anti-bacterial effects of plum fruits grown at lower altitude were found stronger. Inhibition zones which were measured as 0.0, 7.2, and 8.0 at high altitude, against *Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus* bacterial strains respectively, increased to 14.1, 30.8 and 32.9 levels at low altitude. Measured values at low altitude were found to be more effective than vancomycin, cefepime and levoflaxacin antibiotics used as reference. Anti-fungal effects of fruits were shown variability among the fungi. While fruits that were harvested from high altitude were determined more effective against *Candida albicans*, the opposite situation was observed for *Candida parapisilosis*. Fruits harvested from altitude which was more effective against to fungi were shown higher anti-fungal capacity than fluconazole fungicide used as a reference.

Table 2. Phytochemical and antimicrobial characteristics of ‘Black Diamond’ according to altitudes

General Phytochemical characteristics of ‘Black Diamond’			
	pH	Total phenol (mg L^{-1})	Antioxidant activity (%)
B.Diamond (A:200)	3.67 a	339.8 b	32.3 b
B.Diamond (A:800)	3.39 b	817.8 a	74.5 a

Organic acid composition of ‘Black Diamond’ (ppm)				
	Malic acid	Acetic acid	Oxalic acid	Ascorbic acid
B.Diamond (A:200)	6164.3 b	3984.5 a	914.65 b	13.6 b
B.Diamond (A:800)	9628.0 a	2786.5 b	1025.1 a	17.0 a

Phenolic acid composition of ‘Black Diamond’ (ppm)				
	p-hydroxybenzoic acid	p-coumaric acid	Chlorogenic acid	Protocatechuic acid
B.Diamond (A:200)	10.8 b	1.8 b	16.2 a	16.1 ns
B.Diamond (A:800)	21.1 a	2.7 a	1.1 b	20.1

	Gallic acid	Vanillic acid	Caffeic acid	Syringic acid	Routine
B.Diamond (A:200)	14.0 b	7.3 b	7.1 b	5.8 b	1.3 b
B.Diamond (A:800)	21.0 a	17.2 a	21.5 a	9.8 a	3.6 a

Antimicrobial activities of ‘Black Diamond’ (mm)					
	Escherichia coli	*Enterococcus faecalis*	*Staphylococcus aureus*	*Candida albicans*	*Candida parapisilozis*
B.Diamond (A:200)	14.1 a	33.5 a	18.5 a	6.5 b	14.3 a
B.Diamond (A:800)	0.0 b	7.2 b	8.0 b	13.3 a	0.0 b
Vancomycin (Antibiotic)	14.1	30.8	32.9	-	-
Cefepime (Antibiotic)	13.6	13.7	12.9	-	-
Levoflaxacin (Antibiotic)	13.9	28.8	14.7	-	-
Fluconazole (Fungicide)	-	-	-	12.4	8.4

ns, represents non-significance at P<0.05; different letters for each characteristics in columns mean statistical difference among locations at P<0.05.

It is stated that plant compounds show strong anti-microbial effects in microorganisms by disrupting some mechanisms such as nucleic acid synthesis inhibition, interaction with some important enzymes, cytoplasmic membrane function inhibition, polypeptide reaction and biofilm formation (Gonelimali et al., 2018; Mickymaray, 2019). Due to their rich and diverse phytochemical composition, some fruit species, including plum, were reported to have a high anti-microbial effect (Yilmaz and Ercisli, 2011; Sirdaarta et al., 2015; Erbil et al., 2018).

Correlation coefficients between phytochemical characteristics and activity of microorganisms are given in Table 3. According to the results, bacterias and yeasts were determined to be compatible with each other in their own groups.

Table 3. Correlation coefficients between phytochemical characteristics and activity of microorganisms

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	-0.99	-0.99	0.78	-0.81	-0.99	-0.92	0.99	-0.99	-0.97	0.99	-0.81	-0.91	-0.65	-0.89	-0.95	-0.83
2	-0.98	-0.99	0.81	-0.82	-0.99	-0.94	0.99	-0.99	-0.97	0.99	-0.80	-0.93	-0.64	-0.89	-0.95	-0.82
3	-0.98	-0.99	0.82	-0.82	-0.99	-0.94	0.99	-0.98	-0.96	0.99	-0.78	-0.93	-0.62	-0.88	-0.95	-0.80
4	0.99	0.99	-0.76	0.78	0.99	0.90	-0.98	0.99	0.97	-0.99	0.79	0.91	0.63	0.89	0.93	0.80
5	0.99	0.99	-0.78	0.80	0.99	0.91	-0.99	0.99	0.98	-0.99	0.81	0.94	0.64	0.89	0.94	0.81

In columns, 1: *Escherichia coli*, 2: *Enterococcus faecalis*, 3: *Staphylococcus aureus*, 4: *Candida albicans*, 5: *Candida parapisilozis*

In rows, 1:Total phenol. 2:Antioxidant activity. 3: pH. 4:Oxalic acid. 5:Malic acid. 6:Ascorbic acid. 7:Acetic acid. 8:Routine. 9:Parahydroxybenzoic acid. 10:Chlorogenic acid. 11:p-coumaric acid. 12:Gallic acid. 13:Protocatechuic acid. 14:Vanilic acid. 15:Caffeic acid. 16:Syringic acid

Yeasts and bacterial strains were reacted to phytochemical compounds in the opposite way. As the presence of oxalic acid, malic acid, ascorbic acid, routine, parahydroxybenzoic acid, p-coumaric acid, gallic acid, protocatechuic acid, vanilic acid, caffeic acid and syringic acid increases, the development of yeasts were declined, while the development of bacterial strains were increased. Conversely, increase in the amount of acetic acid and chlorogenic acid had been shown to inhibit the development of bacterial strains, while increasing the development of yeasts.

Conclusions

In line with the obtained results from the study, it was determined that the phytochemical composition and antimicrobial activity varied parallel to the altitude change within the same latitude. Amount of phenolic compounds and organic acids were increased with altitude increase while antimicrobial activities were decreased. However, since the reaction of each variety will be different, it is useful to carry out such researches with as many varieties as possible.

It is seen that the pH feature has a key role for the increase of phytochemical characteristics. For this reason, low pH or high acidity must be taken into consideration for the development of varieties with superior phytochemical characteristics.

Author contributions

Design of the research was done by Kerem Mertoglu. KM, also made the spectrophotometric analyzes, statistical analyzes, interpretation of the results and writing of the article. İbrahim Bulduk made the HPLC analyzes and the anti-microbial analyzes were conducted by Aysel Gülbandılar.

Conflict of interest

The authors declare that they have no competing interests.

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