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Antioxidant properties and phenolic profile of the most widely appreciated cultivated berry species: A comparative study

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ABSTRACT

Berries are in the form of a semi-bush or shrub, soft fleshy, juicy, often small and edible. They are generally in bright colours and may sweet or sour in taste. The present study reports a comparison of the antioxidant properties and phenolic profile of the most consumed berry species, namely redcurrant (*Ribes rubrum* L.), blackcurrant (*Ribes nigrum* L.), red raspberry (*Rubus idaeus* L.), blackberry (*Rubus fruticosus* L.), gooseberry (*Ribes uva-crispa* L.) and jostaberry (*Ribes* × *nidigrolaria* Rud. Bauer & A. Bauer). Results of the present study suggested that the highest antioxidant activity (426.26 mg · 100 g^{-1}) and anthocyanin contents (226.33 mg · 100 g^{-1}) are found in Heritage variety of the raspberry species. The highest total phenol content was determined as 1,593.92 mg of Gallic Acid Equivalents (GAE) in jostaberry fruits. The highest ascorbic acid content was obtained from the Goliath variety of blackcurrant as 2,659.26 mg · 100 g^{-1}. The highest ellagic acid value was found as 48.30 mg · 100 g^{-1} in gooseberry, the highest fumaric and citric acid concentrations were noted from jostaberry fruits as 121.88 mg · 100 g^{-1} and 14.84 mg · 100 g^{-1}, respectively, while the highest malic acid content was determined as 14.70 mg · 100 g^{-1} in gooseberry fruits. Our results suggest that berries rich in antioxidant properties and phenolic profile may be an important raw plant material for both the pharmaceutical and food industries.

Keywords: antioxidant, berries, blackberries, currant, phenols, raspberry

INTRODUCTION

The most well-known berries are strawberry, raspberry, blackberry, currant, gooseberry, blueberry, rosehip and jackal plum. Some of the species, such as buffaloberry, serviceberry, cloudberry and farkleberry, have poor economic significance. They are located in forest areas or grown as an ornamental plant. However, due to the scientific confirmation of their contribution to human health, the wild-seen species began to be cultivated (Okatan, 2018). The fruits of different berries (i.e. strawberry, currants, blueberries, raspberry, gooseberry, jostaberry, etc.) look similar in terms of fruit characteristics (mostly aggregate fruit), however, the trees have significant differences in terms of morphology, environmental requirements and cultivation techniques. Therefore, the cultivation of different berries together helps to increase the biodiversity and sustainability of agricultural plantations.

Berries are very important among other fruits due to their unique colour, taste and smell, rich vitamin and mineral contents and a wide variety of uses in the food industry. They can be cultivated in regions at extreme conditions where many other fruit species cannot grow. They show a wide distribution in Asia and Europe to the borders of the North Pole, covering the Caucasus and Iran in the South, and all Mediterranean countries. Rich diversification of species is found in the North American continent, especially in the USA and Canada (Okatan and Çolak, 2019; Zheng et al., 2019).

In terms of human health, vitamins E and C, phenolic substances and organic acids are prominent due to

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their antioxidant properties. High antioxidant capacity of berries is due to phenolic substances, especially anthocyanins rather than ascorbic acid (Gündeşli et al., 2019). The human energy demands can be easily stated with the rapid absorption of soluble sugars contained in the berry fruits. Berry species can be consumed fresh or processed. Dark ones, including blackcurrants, chokeberry and blackberry, are known to contain anti-carcinogenic substances (Borges et al., 2010; Pereira et al., 2018). Leaves of berry species contain tannins, flavones, vitamin C, organic acids and sugars. They are also used in tonic, diuretic, wound therapy and diabetes. In addition, they are used in mouthwash for gums, tonsils and throat infections. Berry fruits are astringents and are used internally and in mouthwash (Şar, 2011; Okatan, 2018). Organic acids are easily absorbed by the human digestive system bringing such effects as a strong antioxidant, anti-bacterial and anti-viral activities, the ability to inhibit mutation on DNA, anti-carcinogen and the ability to prevent some types of cancer. In addition, organic acids show strong toxin cleansing and anti-heart attack features (Boehning et al., 2018).

It is obvious that the chemical composition of different berry species may differ from each other. However, it is good to compare the level of basic compounds from plants of one location to underline these differences and to point the most interesting and promising species. Due to the previously described properties of the berry species, the present work highlights differentiation of fruits' antioxidant potential and phytochemical composition, namely total phenol, antioxidant, anthocyanin, ascorbic acid, major phenolic compounds and organic acid contents in redcurrant, blackcurrant, raspberry, blackberry, gooseberry and jostaberry in Turkey.

MATERIALS AND METHODS

Fruit materials

The fruit materials of the present study were harvested from a commercial orchard found in Aksu Village of Kestel District located in Bursa Province in June 2019. Rovada variety for redcurrant, Goliath variety for blackcurrant, Heritage variety for raspberry, wild forms of blackberry, gooseberry and jostaberry were used in this study as plant materials. All plant materials were 7 years old and fruits were harvested from five different plants for each species. The study area is one of the Turkey's most significant agricultural areas and is located at 40°09′56.18″N and 29°17′58.25″E. Harvested fruits were immediately brought to the Laboratory of Çukurova University for chemical analyses. The analyses were done in three replicates and each replicate consisted of 200 g of fruits.

Determination of total phenolic content

The determination of the phenolic content of the fruits was assessed by the spectrophotometric method according to the Folin–Ciocalteu colorimetry (Li et al., 2007). Briefly, 60 mL of pure water was added with 1 mL of fruit extracts. Then, 5 mL of Folin–Ciocalteu ready solution was added and mixed well. After 7.5 min, sodium carbonate solution (15 mL of 20%) was added to the mixture and the volume of the solutions was mixed to 100 mL and stirred. After centrifugation (15,000 *g*, for 10 min at 25°C), final solutions were stored in the dark at 25°C for 2 h. Hereafter, the absorbance of the solutions was read at 750 nm by a spectrophotometer. Total phenol amounts were evaluated from the calibration curve obtained with gallic acid and values to be equivalent to milligrams of gallic acid per gram (mg GAE $\cdot$ g^{-1} dry weight (DW)) (Li et al., 2007).

Antioxidant activity

The antioxidant capacity of the fruit extract as scavenging activity was assessed by the method described by Re et al. (1999). $ABTS^+$ was generated by the reaction of 7 mM aqueous solution of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) with 2.45 mM aqueous solution of potassium persulphate. This solution was then kept in dark condition for 16 h. Then, 90% methanol was used to dilute the $ABTS^+$ solution to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30ºC. Next, 2 mL of $ABTS^+$ was mixed with 20 µL of extracts, and 90% methanol was used as blank for the spectrophotometric measurements. The absorbance was recorded at 734 nm after 6 min at 25 ºC. The results were expressed as micromoles of Trolox (TE) per gram dry weight sample (µmol Trolox Equivalent Antioxidant Capacity $\cdot$ g^{-1} DW).

Determining total anthocyanin

The total anthocyanin contents of fruit samples were calculated by using the absorbance values taken by spectrophotometer at different pH ranges according to the method suggested by Giusti and Wrolstad (2001). For the measurement of the diluted extracts, pH 1.0 (hydrochloric acid–potassium chloride) and pH 4.5 buffer solutions were prepared and the absorbance values were measured at 531 and 700 nm. The total anthocyanin content (molar extinction coefficient of 28,000, cyanidin-3-glucoside) and absorbance [(A531–A700) pH 1.0–(A531–A700) pH 4.5] were calculated for milligrams per 100 g fresh weight.

Analysis of ascorbic acid

Ascorbic acid contents of the fruit samples were determined with high-performance liquid chromatography (HPLC) method proposed by Cemeroglu (2007) and Geçer et al. (2016). Briefly, 5 mL of fruit extracts were mixed with 2.5% (w/v) metaphosphoric acid (Sigma, M6285, 33.5%) and then centrifuged at 5,500 rpm for 15 min at 4°C. Then, 0.5 mL solution was raised to 2.5 mL (w/v) with metaphosphoric acid. The supernatant was then filtered through a 0.45 PTm PTFE syringe filter (Phenomenex, UK). A C18 column (Phenomenex Luna C18, 250 mm × 4.60 mm, 5 mm) was used at 25°C to identify ascorbic acid.

Major phenolic compounds and organic acids

Phenolic acids of the fruit samples were determined via the method of Rodriguez-Delgado et al. (2001). For this purpose, fruit samples were blended with pure water (1:1). Then, the mixture was centrifuged for 15 min at 15,000 rpm. The isolation of phenolic acids was carried out with an Agilent 1260 series HPLC system equipped with on-line degasser (G 1322A), quat pump (G 1311A), autosampler (G 1313A), column heater (G 1316A) and UV detector (G 1315A). Organic acid composition of the fruits was then determined with the methods described by Bevilacqua and Califano (1989) and Geçer et al. (2016) with minor modifications. Extract of fruits was obtained by crushing the fruits in cheesecloth. The 0.009 N H_2SO_4 was then homogenised with shaker for 1 h. The mixture was then centrifuged at 15,000 rpm for 15 min and the supernatants were filtered twice (one by a 0.45 µm membrane filter and second by a SEP-PAK C18 cartridge). Organic acid readings were performed by HPLC using the Aminex column at 214 and 280 nm wavelengths.

Statistical analysis

Raw data of the experiments were subjected to the analysis of variance and the mean separation was performed with Tukey's HSD test at $p < 0.05$. Furthermore, the 'FactoMineR' and 'factoextra' package of *R* were used to perform biplot principal components analysis (PCA) analysis.

RESULTS AND DISCUSSIONS

Total phenolic contents, antioxidant activity, anthocyanin and ascorbic acid contents of the berry species were found to significantly differ at $p < 0.05$ (Table 1). The highest total phenol value was obtained in jostaberry fruits. The lowest total phenol value was obtained in redcurrant fruits. The highest antioxidant level was obtained in blackberry fruits, while the lowest value was obtained in redcurrant fruits. The highest anthocyanin value was obtained in blackberry, while the lowest value was obtained in redcurrant fruits. The highest value of ascorbic acid was found in blackcurrant fruits. The lowest value of ascorbic acid was obtained in raspberry fruits.

In a previous study, Okatan (2016) reported that the total phenolic content of redcurrants and blackcurrants varied between 5.68 and 10.30 mg GAE · 100 g^{-1} FW and 5.27 and 17.17 mg GAE · 100 g^{-1} FW, respectively. Researchers previously determined that the antioxidant content of redcurrants is between 12.67 and 29.29 mg · 100 g^{-1} FW (Villano et al., 2007; Aneta et al., 2013). Anthocyanin value of redcurrants was previously found as 7.5 mg · 100 g^{-1} FW (Chiang et al., 2013). In another study, the ascorbic acid contents of the redcurrants were reported to vary between 35.41 and 1,410.60 mg · 100 g^{-1} FW (Pantelidis et al., 2007; Aneta et al., 2013; Okatan, 2016). Similarly, Mikulic-Petkovsek et al. (2012) found that the antioxidant activity of blackcurrants was 73.55 mg · 100 g^{-1} FW. In another study, Rubinskiene et al. (2005) determined the value of anthocyanin content between 14.65 and 15.42 mg · 100 g^{-1}. In previous studies, researchers determined ascorbic acid values of blackcurrants between 52.97 and 2,779.30 mg · 100 g^{-1} FW (Gerçekçioğlu et al., 2009; Okatan, 2016). Total phenolic acids were found between 113.73 and 1,822 mg GAE · 100 g^{-1} FW in raspberries, between 48.9 and 690.2 mg GAE · 100 g^{-1} FW in blackberries and between 290 and 2,611 mg GAE · 100 g^{-1} FW in gooseberries (Proteggente et al., 2002; Wada and Ou, 2002; Wang and Lin, 2002; Pantelidis et al., 2007; Giovanelli et al., 2012). In other studies, antioxidant activity was found in line with the findings of the present study in fruits of raspberry, blackberry and gooseberry (Wang and Lin, 2002; Barros et al., 2010; Bobinaitė et al., 2012; Laczkó-Zöldi et al., 2018; Chanyotha et al., 2019). Researchers determined the values of anthocyanin in raspberry 15.1–608.24 mg · 100 g^{-1} FW), blackberry (35.1–230.74 mg 100 · g^{-1} FW) and gooseberry (1.3–152.2 mg · 100 g^{-1}) among different amounts (Laleh et al., 2006; Buřičová et al., 2011; Narváez-Cuenca et al., 2014; Kostecka-Gugała et al., 2015). The main reason for the differences between this study and other studies is due to different ecological conditions.

The major phenolic acid compounds were remarkably differentiated in all the berry species at a statistically important level, $p < 0.05$ (Table 2). Rutin value was determined as the highest in jostaberry fruits, and the lowest value was found in redcurrant fruits. Catechin values were found in fruits of blackberry, jostaberry,

Table 1. Comparison of some of the chemical compounds in different berries

Berries species (cultivar)	Total phenol (mg GAE · 100 g^{-1} FW)	Antioxidant (mg · 100 g^{-1} FW)	Anthocyanin (mg · 100 g^{-1} FW)	Ascorbic acid (mg · 100 g^{-1} FW)
Redcurrant (Rovada)	8.45 e	24.41 f	8.70 e	1,326.46 b
Blackcurrant (Goliath)	11.36 e	74.43 d	17.59 e	2,659.26 a
Raspberry (Heritage)	735.03 c	129.25 b	176.11 b	32.26 f
Blackberry (Wild plants)	518.66 d	426.26 a	226.33 a	67.05 e
Gooseberry	1,223.71 b	72.23 e	84.61 d	157.91 d
Jostaberry	1,593.92 a	125.49 c	114.80 c	451.66 c

Values followed by the same letter or letters within the same column are not significantly different at $p < 0.05$ (Tukey's HSD).

gooseberry, raspberry, blackcurrant and redcurrant. Chlorogenic acid was found the highest in blackcurrant fruits. Quercetin was found the highest in blackberries, and the lowest value was determined in redcurrant fruits.

Liao et al. (2015) analysed the effect of anthocyanins on the colour of 'Schisandra Chinensis' grape with multiple regression analysis between colour parameters and anthocyanin components and found that rutin is the main cause of change in fruit colour. In other previous studies with blackcurrants, rutin values ranged between 15.71 and 35.41 mg · 100 g^{-1} FW, catechin value was reported to be 10.24 mg · 100 g^{-1} FW, chlorogenic acid value was reported between 18.35 and 65.49 mg · 100 g^{-1} FW and quercetin value was noted to be between 1.50 and 2.20 mg · 100 g^{-1} FW. In the same study for redcurrants, rutin values ranged between 7.23 and 18.52 mg · 100 g^{-1} FW, catechin value 7.09 mg · 100 g^{-1} FW, chlorogenic acid value 2.38 mg · 100 g^{-1} and quercetin value between 2.01 and 2.29 mg · 100 g^{-1} (Okatan, 2016). A researcher found quercetin value as 6.32 and rutin value as 15.73 in gooseberry fruits (Chiang et al., 2013). In other studies, rutin contents ranged between 0.97 and 22.77 mg · 100 g^{-1} FW and ferulic acid contents between 0.389 and 2.75 mg · 100 g^{-1} in blackberry fruits (Gudej and Tomczyk, 2004; Buřičová et al., 2011; Zia-Ul-Haq et al., 2014; Gundoğdu et al., 2016). The differences in the results are thought to be due to differences in plants, climatic conditions and different ecologies.

The organic acid contents were remarkably differentiated in all the berry species at a statistically important level, $p < 0.05$ (Table 3). Ellagic acid values of species were determined in gooseberry, jostaberry, blackberry, raspberry, blackcurrant and redcurrant fruits. Fumaric acid values of berries were found the highest in fruits of jostaberry. Fumaric acid values of blackberry, gooseberry and raspberry were found the same in statistical analysis. The lowest fumaric acid value was determined in redcurrant fruits. The highest malic acid value was obtained in gooseberry fruits and the lowest value was found in raspberry fruits.

In recent years, it has been found that the presence of ellagic acid is significant in berries. The types and values of ellagic acid calculated according to the weight of 1 g dry fruit are as follows: red raspberry 1.50 mg, strawberry 0.63 mg, walnut 0.59 mg, pecan 0.33 mg and cranberry 0.12 mg (Daniel et al., 1989). Researchers found ellagic acid values between 7.7 and 19.7 mg · 100 g^{-1}, catechin values between 6.5 and 42.43 mg · 100 g^{-1}, gallic acid between 3.00 and 14.6 mg · 100 g^{-1} and ferulic acid values between 1.00 and 4.90 mg · 100 g^{-1} in raspberry fruits (Pantelidis et al., 2007; Pelc et al., 2009; Gulcin et al., 2011; Gevrenova et al., 2013; Adina et al., 2017). In other studies, catechin contents ranged between 111.60 and 438.97 mg · 100 g^{-1}, ellagic acid contents between 10.61 and 51.51 mg · 100 g^{-1}, gallic acid contents between 2.20 and 9.43 mg · 100 g^{-1} and caffeic acid contents between 1.16 and 12.90 mg · 100 g^{-1} in blackberry fruits (Gudej and Tomczyk, 2004; Buřičová et al., 2011; Zia-Ul-Haq et al., 2014; Gundoğdu et al., 2016). Caffeic acid value was determined as 2.22 mg · 100 g^{-1}, kaempferol value as 28.98 mg · 100 g^{-1} and *p*-coumaric acid value as 6.99 mg · 100 g^{-1} in gooseberry fruits (Chiang et al., 2013). Research has indicated that bioactive values of berries are affected by soil structure, climatic factors, genetic factors and climate. While some results of this study are in agreement with the findings of other researchers, some results are not similar. The differences are thought to be due to different ecological conditions, different harvest times and genetic differences.

The PCA biplot analysis made it possible to evaluate the berry species in terms of the all tested biochemical

Table 2. Comparison of the major phenolic acid compounds in different berries (mg · 100 g^{-1} FW)

Species (cultivar)	Rutin	Catechin	Chlorogenic acid	Quercetin
Redcurrant (Rovada)	13.46 e	7.62 f	2.24 f	2.17 e
Blackcurrant (Goliath)	15.59 d	11.37 e	56.77 a	2.47 e
Raspberry (Heritage)	18.32 b	43.15 d	11.71 e	4.16 d
Blackberry (Wild plants)	17.19 c	327.90 a	22.57 d	8.46 a
Gooseberry	15.53 d	124.64 c	35.20 c	6.16 c
Jostaberry	22.29 a	164.64 b	42.56 b	7.30 b

Values followed by the same letter or letters within the same column are not significantly different at $p < 0.05$ (Tukey's HSD).

Table 3. Comparison of the organic acids contents in investigated berries (mg · 100 g^{-1} FW)

Species	Ellagic acid	Fumaric acid	Citric acid	Malic acid
Redcurrant (Rovada)	4.85 e	7.29 d	13.44 b	2.55 d
Blackcurrant (Goliath)	5.76 e	8.98 c	12.13 c	7.14 c
Raspberry (Heritage)	11.35 d	32.47 b	9.60 d	0.98 e
Blackberry (Wild plants)	37.43 c	33.53 b	5.27 e	2.05 d
Gooseberry	48.30 a	33.35 b	9.31 d	14.70 a
Jostaberry	39.47 b	121.88 a	14.84 a	13.50 b

Values followed by the same letter or letters within the same column are not significantly different at $p < 0.05$ (Tukey's HSD).

compounds. It is clear from Figure 1 that the gooseberry and jostaberry species have the highest concentrations of chlorogenic, malic acid, fumaric acid, total phenolic, rutin and ellagic acid. Blackberries (wild plants) are found to be high in quercetin, catechin and anthocyanins and showed the highest antioxidant activity. The blackcurrant ('Goliath') species was found to be superior in terms of ascorbic acid concentration while all other biochemical compounds were lowest in this species. The other two species of the present study, the redcurrant ('Rovada') and raspberry ('Heritage') are found to be the poorest sources of the biochemical compounds. The results of PCA analysis also made it possible to correlate the biochemical compounds. According to the results, some of the chemicals were found to have strong positive correlations (chlorogenic vs. malic acid; fumaric acid vs. TP (Total Phenol); fumaric acid vs. rutin; TP vs. rutin; rutin vs. ellagic acid; ellagic acid vs. quercetin; quercetin vs. catechin; catechin vs. ACNs; ACNs vs. AA) and some strong negative correlations (AsA (Ascorbic Acid) vs. AA; AsA vs. ACNs; AsA vs. Catechin; AsA vs. quercetin; citric acid vs. AA; citric acid vs. ANCs (Acid Neutralizing Capacity); citric acid vs. catechin) where some of them are not correlated (AsA vs. chlorogenic; AsA vs. malic acid; citric acid vs. fumaric acid; citric acid vs. TP; citric acid vs. rutin; chlorogenic vs. quercetin; malic acid vs. quercetin; fumaric acid vs. catechin; TP vs. ACNS; TP vs. AA; rutin vs. ACNs; rutin vs. AA). The highest correlation was obtained among the total phenolic contents with fumaric acid, rutin and ellagic acid. Moreover, malic acid and chlorogenic acid were also noted to have a strong correlation. The anthocyanin content, as a source of antioxidant activity, was also strongly correlated with antioxidant activity.

CONCLUSION

In this study, chemical compounds of the berry species were determined, and nutritional values and importance of berry species for human health were found due to its large amount of bioactive components. Jostaberry fruits have the highest antioxidant activity among the fruit species studied. The highest ascorbic acid content was found in blackcurrant ('Goliath') fruits. The highest value of rutin was found in jostaberry. The highest values of ellagic acid and malic acid were determined in gooseberry fruits. Berries are very valuable fruits and they contain high levels of bioactive substances. Therefore, the cultivation of these fruit species should be expanded. It is noteworthy that gooseberry and jostaberry are rich in organic acids.

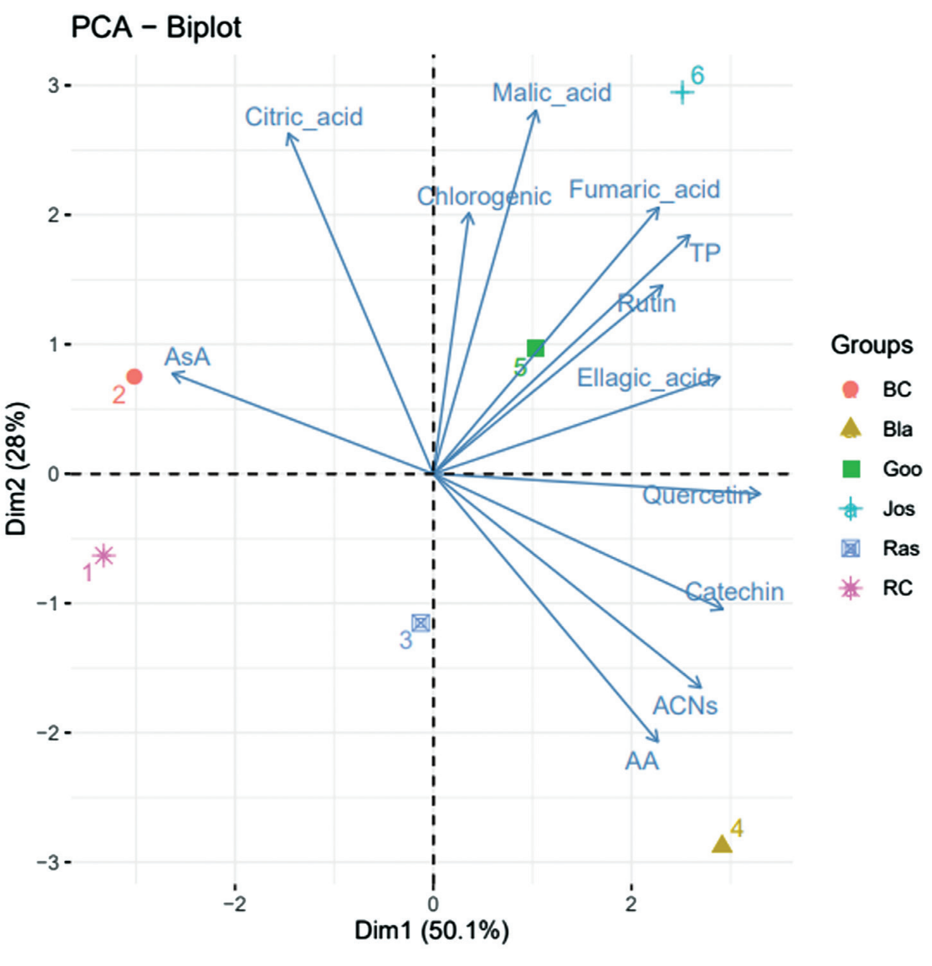

Figure 1. PCA biplot analysis of the berry species and chemical compounds.

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CONFLICT OF INTEREST

The author declares that no conflict of interest exists.

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