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## Phytochemical profiles and antioxidant activity of some grape accessions (*Vitis* spp.) native to Eastern Anatolia of Turkey

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### Summary

Phytochemical profiles and antioxidant activity of four historical grape accessions ('Kuzu Kuyruğu', 'Miskali', 'Erkek Miskali', and 'Kirmizi Kismis') grown in Igdir province located in Eastern Anatolia Region of Turkey were examined. The amount of vitamin C, organic acids (citric acid, tartaric acid, malic acid, succinic acid and fumaric acid), sugars (fructose and glucose), phenolic acids (catechin, rutin, quercetin, chlorogenic acid, ferulic acid, o-coumaric acid, p-coumaric acid, caffeic acid, syringic acid, vanillic acid, and gallic acid), and antioxidant capacity (Trolox Equivalent Antioxidant capacity, TEAC assay) were determined. Accession was found to be important source of variation for all the parameters identified above ( $P < 0.01$ ). Among the grape accessions analyzed, 'Kuzu Kuyruğu' had the predominant vitamin C (47.19 mg/100 g), chlorogenic acid (2.687 mg/L), ferulic acid (1.303 mg/L), o-coumaric acid (1.317 mg/L), and syringic acid content (1.687 mg/L). The highest citric acid (0.873 g/L), fructose (10.36 g/100g), glucose (11.51 g/100g), and catechin (1.353 mg/L) were recorded in 'Miskali' accession. 'Kirmizi Kismis' was determined to be the accession with the highest tartaric acid (10.80 g/L), succinic acid (0.94 g/L), and caffeic acid (2.137 mg/L) levels. 'Erkek Miskali' accession produced the paramount contents for fumaric acid (0.0042 g/L), rutin (2.477 mg/L), quercetin (0.447 mg/L), and vanillic acid (0.313 mg/L). The investigated grape accessions showed notable levels of sugars, organic acids and phenolic compounds. These accessions could be valuable in breeding programs for improving grape quality and nutrition, as well as enhancing commercial worth and production of the grapes in Igdir province of Turkey.

### Introduction

Horticultural plants including fruits, vegetables and grapes have long been valued as part of a nutritious and tasty diet. The flavours provided by different horticultural species are among the most preferred in the world, and it is increasingly evident that horticultural plants not only taste good, but are also good for human health (BACVONKRALJ et al., 2014; ROP et al., 2014). It is well established that horticultural plants and products are a rich source of vitamins, organic acids, sugars, phenolics, minerals and dietary fibre (non-starch polysaccharides) that are essential for normal growth and development and overall nutritional well-being (ERCISLI and ESITKEN, 2004; HEGEDUS et al., 2010).

Grapes are among the most economically important horticultural crops with a total worldwide production of 67 000 000 MT. China is the leading producer followed by USA. Turkey ranks 6<sup>th</sup> in the world in total grape production and has the 4<sup>th</sup> largest vineyard area in the world after Spain, France and Italy (FAO, 2012).

Grapes are classified as table grapes, wine grapes, raisin grapes and juice grapes (PATIL et al., 1995). Especially in the fresh market,

flavor is the most important quality criterion, mainly determined from the ratios of organic acids and sugars, which are also responsible for the taste of the fruit. Tartaric, malic, citric, and succinic acid are among the most important organic acids, not exceeding the total juice weight more than 1 %. Differences in organic acids content in grapes are generally associated with genotype, maturity, environmental conditions and other factors (SOYER et al., 2003; ROBREDO et al., 2011). Other important chemical components of the grape are the polyphenols, well-known for their beneficial effects on human health due to their antioxidant activity (XIA et al., 2010). Anthocyanins, flavonoids and phenolic acids are among the main phenolic compounds (SPACIL et al., 2008). Anthocyanins are pigments corresponding for the color of the fruit. Flavonoids are mainly formed as catechin, epicatechin and procyanidin polymers. Gallic, caffeic, chlorogenic, catechin, epicatechin are among the most important phenolic acids (XIA et al., 2010; SANTOS et al., 2011). Many reports are available about the beneficial effects of phenolic compounds on human health as inhibiting cardiovascular diseases and cancer, as well as slowing aging (TSANGA et al., 2005; GOD et al., 2007; SHANMUGANAYAGAM et al., 2007). Grapes are also very rich source in vitamins, minerals, carbohydrates and fibers, which are key components in human nutrition (XIA et al., 2010).

Factors like genotype and environmental conditions have influence on the chemical composition and antioxidant activity of grapes (LIMA et al., 2014). In literature, there were several reports about the extraction and detection of sugars, organic and phenolic acids of the divergent grape cultivars grown under different ecological conditions in Turkey (ORAK et al., 2007; SABIR et al., 2010), but no published knowledge is currently available for historical grape accessions cultivated in the Igdir province of Turkey.

Therefore, the current study was conducted with the objective to determine the organic acid, sugar and phenolic acids and the antioxidant capacities of *Vitis vinifera* grape accessions grown in Kadikislak village of Igdir province of Turkey. Knowledge of phytochemical content and antioxidant activity of the historical accessions will be useful for the progress of the food industry related to grape production in Igdir province of Turkey neighboring on Armenia, Nakhcivan, and Iran, and more specifically will provide a precious reference for further researches who are interested in the related topics.

### Materials and methods

#### Plant material

Accessions 'Kuzu Kuyruğu', 'Miskali', 'Erkek Miskali', and 'Kirmizi Kismis' were used in this study. The berry colour of 'Kuzu Kuyruğu' and 'Erkek Miskali' was white, the other accessions had red berry colour. The collection site is located in the village of Kadikislak village. The village has continental climate with latitude 40° 2' 60 N, longitude 44° 4' 60 E, and altitude 858 meters. Fruits (berries) for analyses were collected from one bunch with

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8 grapevine plants ensuring representative samples per accessions. Berries of those grape accessions were taken during commercial ripening stage varying between 1 to 20 August of the year 2013. Thus, the sampling was structured according to different ripening stages across the individual accessions. The plants were established with a planting scheme of 2.5 m x 2.5 m in distance. During winter months, the plants were covered by soil to avoid winter injury. Pruning was applied in Feb-ruary and 3-4 buds were left on shoots.

#### Analysis of organic acids

The juice of the grapes was extracted by hand mashing using cheese-cloth, and stored at (-20 °C) until analyzed for the following organic acids of succinic, citric, malic, fumaric and tartaric. Aforementioned organic acids were extracted according to the modified method of BEVILACQUA and CALIFANO (1989). Each sample of 5 mL was blended with 20 ml 0.009 N H<sub>2</sub>SO<sub>4</sub> (Heidolph Silent Crusher M, Germany). For homogenization, the mixtures were stirred with a shaker (Heidolph Unimax 1010, Germany) for 1 hour, afterwards centrifuged for 15 min at 15000 g. Following initial filtration with filter paper and twice with membrane filter of 0.45 µm (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), the supernatants were run through SEP-PAK C<sub>18</sub> cartridge. Using Aminex column (HPX - 87 H, 300 mm x 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA), HPLC was commanded by Agilent package program with a DAD detector (Agilent, USA) adjusted to 214 and 280 nm wavelengths.

#### Analysis of phenolic compounds

The following phenolic compounds of gallic acid, catechin, chlorogenic acid, caffeic acid, p-coumaric acid, o-coumaric acid, ferulic acid, vanillic acid, rutin, syringic acid and quercetin were extracted and determined according to the modified method of RODRIGUEZ-DELGADO et al. (2001). The samples were diluted with distilled water in a ratio of 1:1, and centrifuged for 15 min at 15000 g. Following initial filtration with filter paper and twice with 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), the supernatants were passed through HPLC. Chromatographic analysis was performed by Agilent 1100 (Agilent) HPLC system using DAD dedector (Agilent, USA) with 250 x 4.6 mm, 4µm ODS column (HiChrom, USA). As a mobile phase, Solvent A methanol:acetic acid:water (10:2:28) and Solvent B methanol:acetic acid:water (90:2:8) were used, with a flow rate of 1mL/min and 20 µL injection volume for spectral measurements at 254 and 280 nm.

#### Sugar analysis

The sugar analysis was performed using the modified method of MELGAREJO et al. (2000). Standards of fructose and glucose were used. Homogenized samples of 5 ml were centrifuged for 2 min at 12000 g for 2 min and filtered, afterwhich run through SEP-PAK C<sub>18</sub> column. µbondapak-NH<sub>2</sub> column with 85 % acetonitrile liquid phase was used in the HPLC device having a refractive index detec-

tor. Calculation of the sugar concentrations was done based on fruit juice standards.

#### Analysis of ascorbic acid

5 ml juice of each sample from the fruit was transferred to test tubes and mixed with 2.5 % M-phosphoric acid solution. The mixture was centrifuged for 10 min at 6500 rpm and 4 °C. 0.5 ml from the clear part of the blend was complemented to 10 ml using % 2.5 M-phosphoric solution. Afterwards filtered by 0.45 µm teflon filter and injected to HPLC. C<sub>18</sub> column (Phenomenex Luna C<sub>18</sub>, 250 x 4.60 mm, 5 µ) was used in the HPLC device, and temperature was adjusted to 25 °C. Ultra distilled water with 1 ml/min flow rate and pH of 2.2 adjusted with H<sub>2</sub>SO<sub>4</sub> was used as a mobile phase. The readings were made with DAD detector at 254 nm wavelength. For determination of ascorbic acid, different concentration levels of L-ascorbic acid (SigmaA5960) (50, 100, 500, 1000, and 2000 ppm) were used (CE-MEROGLU, 2007).

#### Determination of Trolox Equivalent Antioxidant Capacity (TEAC)

For a standard TEAC measurement, ABTS acetate was prepared with potassium persulphate by dissolving in a buffer (OZGEN et al., 2006). To keep the stability of the mixture for a long time, ABTS<sup>+</sup> was diluted with 20 mM sodium acetate buffer at pH of 4.5, with absorbance of 734 nm, 0.700 ± 0.01. For spectrophotometric measurements, 3 ml ABTS<sup>+</sup> solution was mixed with 20 µl fruit extract, and incubated for 10 min. Absorbance values were obtained at 734 nm.

#### Statistical Analysis

Eight grapevine plants for each accession were used for sampling. Fruits (berries) were collected from different bunch with 8 plants per accession. The descriptive statistics for all quantitative characteristics under investigation were expressed as mean ± SE. The data were analyzed through One-Way ANOVA with four replications and mean separation was done by using Ducan multiple comparison tests. In the present paper, a significance level of 1 % was considered. All statistical computations were performed using a SPSS 20 programme.

## Results and discussion

The current study is a preliminary evaluation report on identification of some organic acids, sugars, and phenolic acids and antioxidant activity of native historical four grape accessions grown in Kadikislak village of Igdır province of Turkey.

Results of statistical analysis in terms of organic acids from the local grape accessions in Kadikislak village of Igdır province of Turkey are summarized in Tab. 1. Results of analysis of variance revealed apparently in Tab. 1 that the accession was a prominent factor for organic acids in the current study (P<0.01). The current finding on organic acids was compatible with those reported in pre-

**Tab. 1:** Organic acids content in berries of grape accessions.

	Citric Acid g/L	Tartaric Acid g/L	Malic Acid g/L	Succinic Acid g/L	Fumaric Acid g/L
'Kuzu Kuyrugu'	0.780±0.02b	8.63±0.26b	3.02±0.23a	0.44±0.01c	0.0036±0.01b
'Miskali'	0.873±0.01a	4.30±0.20d	2.17±0.11b	0.63±0.02b	0.0013±0.01c
'Erkek Miskali'	0.298±0.01d	5.63±0.16c	2.32±0.17b	0.44±0.01c	0.0042±0.02a
'Kirmizi Kismis'	0.345±0.02c	10.80±0.19a	3.59±0.19a	0.94±0.02a	0.0036±0.01b

The difference between the means with different letters in same column was significant (P<0.01)

vius published studies (SOYER et al., 2003; ROBREDO et al., 2011; LIANG et al., 2014). At harvest, the differences in terms of the organic acids may be attributed to cultivars, environmental conditions, and storage time (ROBREDO et al., 2011). ROBREDO et al. (2011) mentioned that the sugar and organic acids composition determine the flavor of the table grapes, which is a very important quality criterion in fresh market. Also, they informed that organic acids provide contribution to the sourness of the fruits. The organic acids were also reported to be a significant indicator on maturity of the grapes that is an important factor for identification of the proper harvesting time (SOYER et al., 2003). The order of the present accessions for citric acid was 'Miskali' > 'Kuzu Kuyruğu' > 'Kirmizi Kismis' > 'Erkek Miskali'. According to the current results, 'Kirmizi Kismis' was determined to be the prominent accession in tartaric acid. The order of the statistically significant differences for tartaric acid was 'Kirmizi Kismis' > 'Kuzu Kuyruğu' > 'Erkek Miskali' > 'Miskali'. For malic acid, the statistical order with respect to statistical test was found as 'Kirmizi Kismis' = 'Kuzu Kuyruğu' > 'Erkek Miskali' = 'Miskali'. As understood from Tab. 1, corresponding accession orders for succinic and fumaric acids were 'Kirmizi Kismis' > 'Miskali' > 'Kuzu Kuyruğu' = 'Erkek Miskali', and 'Erkek Miskali' > 'Kuzu Kuyruğu' = 'Kirmizi Kismis' > 'Miskali', respectively. Composition of organic acids was very important in the grape flavor, and the composition influenced by cultivars should be identified (ROBREDO et al., 2011; SOYER et al., 2003). SABIR et al (2010) reported varied ranges of tartaric acid, malic acid and citric acid of five grape cultivars as 3.8-5.2 g/L, 2.8-3.6 g/L and 0.2-0.4 g/L, respectively. For Red Globe, Thompson Seedless, and Crimson Seedless table grapes usually grown in inner western Anatolia, ROBREDO et al. (2011) recorded 1.28, 2.05 and 1.80 g/L for tartaric acid content; 0.39, 1.80, and 0.51 g/L for malic acid content, respectively. BURIN et al. (2010) notified to be the ranges of 11.64-17.15 g/L for tartaric acid levels of commercial grape juices. The differences may be ascribed to the common effect of accession because all accessions were grown at a single location applying similar cultivation practices. Results of statistical assessments made for antioxidant activity (TEAC assay), vitamin C, fructose and glucose are presented in Tab. 2. As depicted in Tab. 2, the analysis results of variance illustrated that the accession is of big importance in characterizing TEAC, vitamin C, fructose, and glucose levels ( $P < 0.01$ ). The highest contents for TEAC were statistically enabled by 'Kuzu Kuyru-

ğu' = 'Kirmizi Kismis', followed by 'Erkek Miskali' > 'Miskali'. BURIN et al. (2010) notified that there were 2.51-11.05 mM for TEAC levels of commercial and grape juices. However, the predominant content for vitamin C was yielded with 'Kuzu Kuyruğu' accession compared to other accessions used in this study. The statistical significance order of the grape accessions for vitamin C was in the form of 'Kuzu Kuyruğu' > 'Erkek miskali' > 'Miskali' > 'Kirmizi Kismis'. The accession significance order was obtained for fructose, 'Miskali' > 'Erkek miskali' > 'Kirmizi Kismis' = 'Kuzu Kuyruğu' (Tab. 2). Significance order of the accessions in glucose content was noted as 'Miskali' > 'Erkek miskali' > 'Kuzu kuyruğu' > 'Kirmizi Kismis'. For Red Globe, Thompson Seedless, and Crimson Seedless table grapes, ROBREDO et al. (2011) reported, at harvest time, 8.74, 8.05, and 7.74 g/100 g for fructose content, 8.06, 8.71, and 8.03 g/100 g for glucose content, respectively. The previous results of ROBREDO et al. (2011) were higher markedly than the glucose and fructose contents of 'Kuzu Kuyruğu', 'Erkek Miskali', and 'Kirmizi Kismis' accessions evaluated in the current study, but lower than corresponding contents of 'Miskali' accession. Additionally, several authors have reported previously that glucose and fructose contents were available in similar amounts (SABIR et al., 2010; ROBREDO et al., 2011). The mean vitamin C contents from other accessions with the exception of 'Kirmizi Kismis' accession were not in agreement with the content reported by SOUSA et al. (2014). The differences could be related to the individual genetical background or environmental influences. In the study of SABIR et al. (2010), it was obtained that the discriminating ranges were 73.1-94.1 g/L and 86.4-107.0 g/L for fructose and glucose sugars, respectively, which was very higher when made comparison with the corresponding ranges (54.8-83.9 g/l and 50.9- 89.9 g/L and) of RUSJAN and KOROSKORUZA (2007) reported for 15 red wine cultivars. Results of statistical analysis for 11 phenolic compounds are summarized in Tab. 3 and 4, respectively. ANOVA results represented that accession had a significant influence on the studied compounds notified in Tab. 3 and 4 ( $P < 0.01$ ). In terms of catechin content, the highest content was obtained by 'Miskali' accession (1.353 mg/L), but the lowest content by 'Kirmizi Kismis' accession. The accession significance order for catechin content was 'Miskali' > 'Erkek Miskali' > 'Kuzu Kuyruğu' > 'Kirmizi Kismis'. Rutin content of the grapes in the current study ranged from 0.950 to 2.477 mg/L. With regard to Duncan results, the statistical significance order of

**Tab. 2:** Content of vitamin C, fructose and glucose and trolox equivalent antioxidant capacity (TEAC) in berries of grape accessions.

	TEAC ( $\mu\text{mol TE g}^{-1}$ )	Vitamin C mg/100 g	Fructose g/100 g	Glucose g/100 g
'Kuzu Kuyruğu'	8.15±0.392a <sup>y</sup>	47.19±0.30a	5.37±0.15c	6.55±0.25c
'Miskali'	3.35±0.159c	16.17±0.21c	10.36±0.17a	11.51±0.25a
'Erkek Miskali'	6.29±0.332b	23.62±0.38b	6.59±0.22b	7.34±0.17b
'Kirmizi Kismis'	7.29±0.190a	9.85±0.31d	5.43±0.16c	5.86±0.06d

The difference between the means with different letters in same column was significant ( $P < 0.01$ )

**Tab. 3:** Phenolic compounds in berries of the grape accessions.

	Catechin mg/L	Rutin mg/L	Quercetin mg/L	Chlorogenic Acid mg/L	Ferulic Acid mg/L
'Kuzu Kuyruğu'	0.680±0.006c <sup>y</sup>	0.950±0.012c	0.337±0.018b	2.687±0.015a	1.303±0.043a
'Miskali'	1.353±0.012a	2.270±0.090b	0.193±0.009c	2.307±0.049b	0.057±0.003d
'Erkek Miskali'	1.073±0.026b	2.477±0.041a	0.447±0.020a	0.947±0.018c	0.617±0.019b
'Kirmizi Kismis'	0.347±0.012d	1.097±0.041c	0.220±0.012b	0.743±0.019d	0.327±0.009c

The difference between the means with different letters in same column was significant ( $P < 0.01$ )

**Tab. 4:** Phenolic compounds in berries of the grape accessions.

	o-coumaric acid mg/L	p-coumaric acid mg/L	Caffeic acid mg/L	Syringic Acid mg/L	Vanillic Acid mg/L	Gallic Acid mg/L
'Kuzu Kuyruğu'	1.317±0.055a <sup>y</sup>	0.067±0.003b	0.953±0.015b	1.687±0.020a	0.087±0.003c	1.113±0.032a
'Miskali'	1.127±0.027b	0.090±0.000b	0.783±0.044c	0.793±0.027c	ND	0.603±0.042b
'Erkek Miskali'	0.443±0.032d	0.240±0.015a	0.960±0.055b	0.980±0.035b	0.313±0.017a	1.133±0.045a
'Kirmizi Kismis'	0.597±0.024c	0.243±0.015a	2.137±0.048a	0.317±0.039d	0.143±0.009b	0.303±0.027c

The difference between the means with different letters in same column was significant (P<0.01) ND:Non determined

the accessions for rutin content was assigned as 'Erkek Miskali' > 'Miskali' > 'Kirmizi Kismis' = 'Kuzu Kuyruğu'. As perceived expressly from Tab. 3, 'Erkek Miskali' was the grape accession containing the maximum quercetin content. As to Tab. 3, it was clear that the accession with the maximum content in chlorogenic acid was found to be 'Kuzu Kuyruğu', followed statistically by 'Miskali' > 'Erkek Miskali' > 'Kirmizi Kismis' with the range of 0.743 to 2.687 mg/L, respectively. For ferulic acid content with the range of 0.057 to 1.303 mg/L, the order of the grape accessions was 'Kuzu Kuyruğu' > 'Erkek Miskali' > 'Kirmizi Kismis' > 'Miskali' (Tab. 3). The present results from Tab. 3 were quite lower compared to those of LIMA et al. (2014), who obtained the ranges in catechin (4.7-21.0 mg/L), and chlorogenic acid (2.1-21.3 mg/L), for new Brazilian grape cultivars, respectively. But, the findings were in disagreement with the ranges of rutin (0.9-5.9 mg/L), and quercetin (0.5-0.8 mg/L) reported by LIMA et al. (2014). For Emir, Kalecik karası, and Narince grape cultivars, BAYDAR (2006) confirmed 0.28, 18.95, and 1.08 µg/g for rutin content; 0.35, 0.87, and 0.60 µg/g for quercetin content; 0.20, 0.21, and ND for ferulic acid content, and 0.22, ND, and 2.26 for chlorogenic acid content, respectively (ND=Not Detected).

The highest content of o-coumaric acid in the current investigation was recorded for 'Kuzu Kuyruğu' accession and the significance order for o-coumaric acid of the accessions assessed herein was 'Kuzu Kuyruğu' > 'Miskali' > 'Kirmizi Kismis' > 'Erkek Miskali' as also seen in Tab. 4. The means of o-coumaric acid content obtained for the inspected accessions were lower noticeably than those of the p-coumaric acid contents obtained for the accessions (Tab. 4). For the p-coumaric acid content, the accession significance order verified statistically in Tab. 4 was indicated as 'Kirmizi Kismis' = 'Erkek Miskali' > 'Miskali' = 'Kuzu Kuyruğu'. As also depicted in Tab. 4, within the accessions, 'Kirmizi Kismis' with the mean of 2.137 mg/L had the uppermost level for caffeic acid content with the statistical accession order, 'Kirmizi Kismis' > 'Erkek Miskali' = 'Kuzu Kuyruğu' > 'Miskali'. 'Kuzu Kuyruğu' accession produced the prominent syringic acid content with 1.687 mg/L, but the lowest content of 0.317 mg/L was extracted from 'Kirmizi Kismis' grape accession. From Tab. 4, the accession order in syringic acid was statistically detected as 'Kuzu Kuyruğu' > 'Erkek Miskali' > 'Miskali' > 'Kirmizi Kismis'. The vanillic acid content of 'Miskali' amongst accessions was not quantifiable, and the statistically significance order was given as, 'Erkek Miskali' (0.313 mg/L) > 'Kirmizi Kismis' (0.143 mg/L) > 'Kuzu Kuyruğu' (0.087 mg/L) in Tab. 4. The order was formed for gallic acid content, 'Erkek Miskali' = 'Kuzu Kuyruğu' > 'Miskali' > 'Kirmizi Kismis'. The present results from Tab. 4 were higher than those of LIMA et al. (2014), who detected the very wide range for p-coumaric acid (2.1-9.0 mg/L) extracted from new Brazilian grape cultivars, respectively. For three grape cultivars, viz. Emir, Kalecik karası, and Narince, BAYDAR (2006) reported 0.30, 0.46 and 1.22 µg/g for o-coumaric content; 0.13, 0.15, and 0.46 µg/g for p-coumaric content; 0.18, ND, and 0.33 µg/g for caffeic acid content; ND, 0.55, and ND µg/g for syringic acid content; 0.62, 5.20, and 1.30 µg/g for gallic acid content respectively (ND=Not

Detected). The difference may be due to cultivars and environmental variations (BAYDAR, 2006) in terms of phenolic compounds.

## Conclusion

The current study is a first evaluation report on the organic acids, sugars, phenolic compounds and antioxidant capacity of the four grape accessions grown in Kadikislak village of Iğdir province, Turkey. 'Kuzu Kuyruğu' accessions were found to contain very high content of ascorbic acid, chlorogenic acid and ferulic acid and show high antioxidant activity. 'Miskali' and 'Erkek Miskali' were found rich in terms of rutin and catechin. 'Kirmizi Kismis' contained high content of tartaric acid. Therefore, the present results are expected to provide some useful information for further use of promising accessions in grape breeding to obtain high levels of phytochemicals in berries for human health and nutrition.

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