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Research Article

Morphological, quality characteristics, and antioxidant activity of grapes from heritage germplasm grown in Central Anatolia, Turkey

Mustafa OZDEN*, Yasin DEVECI

*Nigde Omer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technologies, 51240 Nigde, Turkey; mozden@ohu.edu.tr (*corresponding author); yasindeveci@hotmail.com*

Abstract

Conservation of Central Anatolian grapevine genetic resources is essential to slow down to loss of biodiversity and genetic resources. This study provided information about fruit quality characteristics including morphological, physical, chemical, and antioxidant potential of ten grape accessions from heritage germplasm grown in Central Anatolia. Physical and chemical quality characteristics measured included berry and cluster weight, must yield proportion, firmness and chroma index, pH, titratable acidity (TA), total soluble solids (TSS), total anthocyanins (TAC), total phenolics (TP) and total flavonoids (TF). Also, antioxidant potential of ethanolic berry extracts were investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) assays. 'Kızıl Üzüm, 'Nuniyar', 'Karaşabı' and 'Kırmızışabı' had higher firmness values and lower must yield, which are important for storage of table grapes. 'Kavak yaprağı' had heaviest berries, highest must yield and TSS/TA ratio giving it excellent flavour. The red grape accession with the highest TAC was 'Kırmızışabı', and the highest TP and TF were recorded for 'Sergi Karası'. Eighty percent of the extracts could scavenge 50% of the DPPH free radical with 0.28 mg mL^{-1} of extract. 'Tilki Kuyruğu' had the highest DPPH free radical scavenging activity due to high phenolic and flavonoid content. FRAP reducing power of extracts also showed a similar trend to DPPH free radical activity. The accessions with rich secondary metabolite profiles inhibited DPPH free radicals and increased FRAP activity. Based on these findings, it is possible to suggest that chemical characteristics including secondary metabolite profile and antioxidant activity of grapes might be used as biochemical marker to discriminate grape cultivars each other. Finally, the region where this study was carried out is very rich in grape diversity, it should be screened and valuable accessions protected for the future.

Keywords: antioxidant potential; chemical characteristics; secondary metabolites; table grapes; *Vitis vinifera* L.

Abbreviations: AE - ascorbic acid equivalent; BHT – butylhydroxytoluene; CE - catechin equivalent; FW - fresh weight, GAE - gallic acid equivalent; TAC - total anthocyanins, TCA - trichloroacetic acid; TF - total flavonoids; TP - total phenolics

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Introduction

The need for conservation, characterization and reintroducing of genetic resources is an important challenge for viticulture. Recently measures and actions taken to preserve existing germplasm diversity have gained urgency in all grape producer countries. Preservation and continual use of grapevine genetic resources depends on the description, characterization and management of germplasm. These measures are needed for germplasm survival and use in breeding, research and production (Margaryan *et al*., 2017).

The geographic origin of the grapevine is considered to be the Transcaucasia region which extends into Georgia, Armenia and Azerbaijan. However, the people of Anatolia, neighboring the Transcaucasia, had a significant role in the domestication and spread of cultivated (*Vitis vinifera* ssp. *sativa*) and wild (*Vitis vinifera* ssp. *sylvestris*) grapevines (Vavilov, 1926; McGovern, 2003). The Anatolian Peninsula (also known as Asia Minor) is located between the Caucasus and Europe, has had crucial source for diversification of grape germplasm around the world due to its geographical location, diverse ecological conditions and long history of viticulture (McGovern, 2003; Ergul *et al*., 2006; Yılmaz *et al*., 2020). Viticulture has been a part of the history, tradition and horticulture of the people living this region. Natural variation and grower selections over centuries have had an important role in the development of biodiversity and led to a strong relationship between genotype and environment. Therefore, the Anatolia region is an important area for diversity and characterization studies of grape genotypes. Characterization should be conducted in this region due to its long history of cultivation of grapevines, diversity in abiotic and biotic stress factors, and variability in organoleptic and nutraceutical features. In fact, these traits are strongly associated with the ecological conditions of geographical areas where vines grow (Gu *et al*., 2002). Plants evolve in response to biotic and abiotic stresses developing biochemical pathways to produce secondary metabolites to protect cells or tissues for survival (Manach *et al*., 2004). Another important concern is that the rich germplasm and genetic resources of the region are under pressure from a move to preferentially grow mostly seedless cultivars. Although preferred by consumers, seedless grape represent a potential loss in health benefits provided by phenolic content of grape seeds (Parry *et al.,* 2006). Given these problems, it is difficult for new table grapes genotypes to be adopted for production and to enter the market. Therefore, the need for characterization and preservation of Central Anatolian grape accessions is increasingly necessary to prevent loss of genetic variability and resources.

Until few years ago, physical quality characteristics of grape berries or clusters including size, colour, shape and weight were the main quality components influencing consumer behaviour. However, revealing the health benefits of grapes through scientific evidence on nutritional properties has been changing consumer behavior (grape selection) towards more nutritional produce (Razvan *et al*., 2017). In addition to vitamins, minerals, sugars and proteins, grapes can have high amounts of phenolic compounds, flavonoids and anthocyanins that provide a wide range of antioxidant activities. Grape secondary metabolites have been demonstrated to have antioxidant (Leifert and Abeywardena, 2008), anti-inflammatory (Dvorakova and Landa, 2017), anti-cancerogenic (Nivelle *et al*., 2017) and antibacterial (Hwang and Lim, 2019) properties, and can modulate the human immune system. For these reasons, there is a growing interest in using these compounds for various aspects of human health and in the food industry (Tako *et al*., 2020). Therefore, the secondary metabolic profile can be used as a biochemical marker for the characterization of grapevine germplasm.

Therefore, this study aimed (1) to provide data to underpin the preservation of some Central Anatolian seeded grape accessions from germplasm at risk of extinction due to intensive use of commercial cultivars,(2) to evaluate the key morphological, physical and chemical quality characteristics including the secondary metabolite contents and antioxidant potential of the accessions, and (3) to introduce these valuable accessions from heritage germplasm in Central Anatolia to breeders and researchers and grape producers who care about the quality characteristics of new genotypes for production in more vineyards.

Materials and Methods

Plant material

Ten seeded grape accessions (*Vitis vinifera*) were selected for study (Figure 1) after the preliminary observation and communication with the local growers and harvested at their commercial maturity stage from the three forty-year-old heritage germplasm vineyards located on Bor District, Niğde Province, Turkey in 2018 vintage.

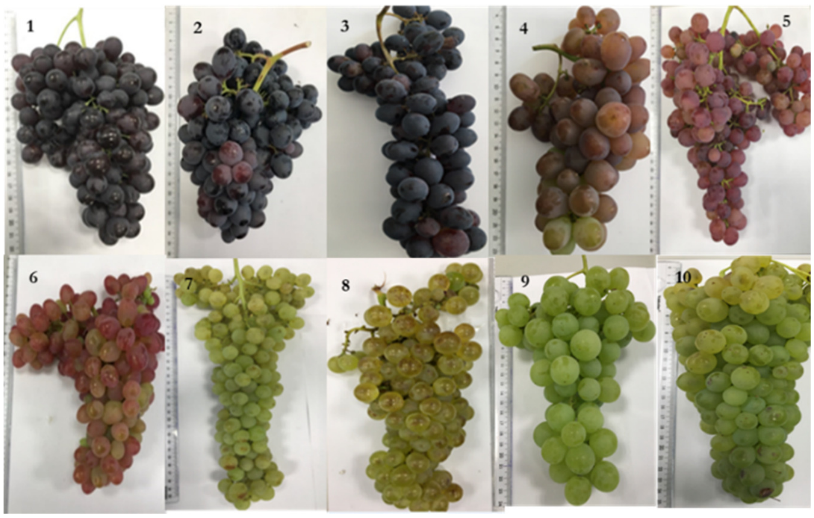

Figure 1. Grape accessions used in the study
1-'Sergi Karası', 2-'Karaşabı', 3-'Kırmızışabı', 4-'Morşabı', 5-'Nuniyar', 6-'Kızıl Üzüm', 7-'Tilki Kuyruğu', 8-'Cevşen', 9-'Kavak Yaprağı', 10-'Dimrit'. Accessions numbered for further works.

Geographical location and growing conditions

Geographical location of the study area was in the middle of Central Anatolia, Turkey at 37.50° N, 34.35° E, and 1,244 m above sea level, with 9.1 °C annual average temperature, 513 mm annual and 55.4 % relative RH as shown in Figure 2. Hot and dry summers with cold and snowy winters is the general climatic pattern of the study area. The soil in the vineyards is calcareous soil with moderately high pH and low organic matter. Although cultural practices of the vineyards were performed regularly by the growers, the vines were not irrigated during the growing season.

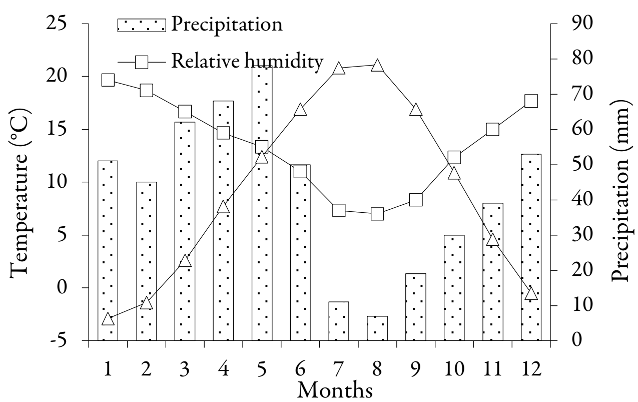

Figure 2. Climate parameters of study area with average temperature (°C), precipitation (mm) and relative humidity (%)

Fruit morphological assessment

According to the International Organization of Vine and Wine (OIV) descriptors, some morphological characteristics of clusters and berries of the accessions were assessed and are presented in Table 1. Each character has an OIV code (Anonymous, 2009) with operational definitions for assessment of the trait. The berries and bunches were morphologically evaluated at commercial maturity. Maturation time was determined based on TSS and TA as well as taste and colour of the accessions. Harvest was made at four occasions between the beginning of August to the end of September. Five berry characteristics (OIV220, OIV221, OIV223, OIV225 and OIV236) and four cluster characteristics (OIV203, OIV204, OIV208 and OIV304) were determined on 20 replicates for each accession. Dimensions of berries and clusters were measured using a digital caliper, and qualitative characters were estimated using the rating and coding according to the OIV descriptors.

Physical measurements

Physical measurements of the berries were made with samples taken from different directions from each grapevine. Cluster weight (g), 100-berry weight (g), must yield (%), berry firmness (N), berry shape index and berry skin colour chroma index were determined as physical measurements. Cluster and berry weight were determined using an analytical balance. Must yield of the accessions were determined based on the OIV descriptor (OIV 233) by weighing of 100 g of berries without pedicels, crushed and centrifuged at 3000 rpm. Firmness of berries were measured by a modification of the method of Aday and Caner (2010) with a texture analyzer (TA.HD.Plus, United Kingdom) as the force (N) required for penetration using a 3 mm probe at a speed of 1 mm s^{-1} and penetration distance of 6 mm. The values were obtained at the same four points on the circumference of each berry. Berry skin colour was measured by the method of McGuire (1992) using a Konica Minolta CR-200B. A single measurement was recorded for each berry and 20 replicate berries were measured for each accession. The value of L* describes the degree of darkness or lightness. Before analysis, a* and b* coordinates were transformed into chroma (*C**) and hue angle (h^o) using the equations: $C^* = (a^{*2} + B^{*2})^{0.5}$ and $h^o = \tan^{-1}(b^*/a^*)$. Richness of colour was represented by C* value.

Chemical measurements and analyses

For the chemical measurements or analyses, grape clusters representing with the vine and vineyard were harvested at commercial maturity according to Rankine *et al.* (1962). Samples were free from visible blemishes or disease. Approximately 30 kg of grape clusters were harvested for each accession, i.e., three 10 kg replicates. Before analyzing, cluster or 100-berry weight of the samples was selected randomly from the clusters for the berry extraction. Berries were homogenized in an ice-cold blender after removal of seeds and a 25 g of the berries were macerated in 100 ml of ethanol containing 0.1% HCl and macerated overnight in darkness. Then the extract was filtered through Whatman No. 1 paper under vacuum and the residue was repeatedly extracted with the same solvent until it was colourless. One part of the extracts was separated for determining anthocyanin content and the remaining portion was concentrated by a rotary evaporator at 50 °C and used for determining total phenolic and flavonoid contents, and antioxidant potential of samples. TA (% tartaric acid), TSS (°Brix) and pH of the grape samples were measured in the fruit juice by standard methods immediately after crushing of the samples. A subsample of 10 g of berry was tissue homogenized in 50 ml of ddH_2O and the liquid level was adjusted to 100 ml for measuring pH (pHenomenal, United Kingdom) and TA. TSS (°Brix) was measured by digital refractometer (Kruss, AR2008, Germany). NaOH solution (0.1 M) was added to the homogenized berry juice until to the endpoint of pH 8.2. TA acidity results were expressed as the percentage of a tartaric acid reference.

TAC content in the grape berry extracts were determined by the pH differential method of Giusti and Wrolstad (2003) using UV spectrophotometer (PG Instruments T60, United Kingdom). Absorbance of the samples in 0.025 M KCl buffer (pH 1.0) and 0.4 M CH_3COONa buffer (pH 4.5) were measured at 520 and 700 nm using $A = (A_{\lambda 520} - A_{\lambda 700})_{pH\,1.0} - (A_{\lambda 520} - A_{\lambda 700})_{pH\,4.5}$. All values were estimated as malvidin-3-glucoside using a molar extinction coefficient of 28,000 kg^{-1} FW (Wrolstad, 1976). TP in ethanolic berry extracts were estimated by the method of Slinkard and Singleton (1977) with minor modifications. Samples (20 µl) were introduced into test tubes, 2,380 mL distilled water and 0.15 mL Folin-Ciocalteu's reagent was added and the tubes were vortexed vigorously. After 8 min, 0.45 mL of Na_2CO_3 was added to each tube, and then each mixture was vortexed again and allowed to stand for 2 h at room temperature. Absorbance of the samples were measured at 750 nm. TP content of the samples were obtained from the calibration curve prepared with gallic acid (20-250 mg L^{-1}) and expressed as mg GAE kg^{-1} FW ($y = 0.0015\ x - 0.123$, $R^2 = 0.99$). TF content of the berry extracts were determined by the modified colorimetric method (Zhishen *et al.*, 1999). TF content obtained from the calibration curve prepared with catechin (20-250 mg L^{-1}) and expressed as mg of CE kg^{-1} FW ($y = 0.0038\ x - 0.0247$, $R^2 = 0.99$)

Antioxidant potential of the accessions

Total antioxidant potential of ethanolic grape berry extracts comprised simply antioxidant activity and antioxidant capacity. Total antioxidant activity of samples was determined by DPPH free radical scavenging activity and expressed as percentage of synthetic free radical inhibition, and total antioxidant capacity was determined by FRAP assay. The DPPH free radical scavenging activity of the ethanolic callus extracts were measured according to DPPH standard method (Blois, 1958). Each ethanolic berry extract (0.1 mL) was added to 2.9 ml of DPPH in ethanol (0.1 mM). The mixture was shaken gently and incubated for 30 min at room temperature. After this period, absorbance was determined at 515 nm in spectrophotometer. Fifty % inhibitory concentration (IC_{50}) values were calculated after constructing the percent inhibition versus log curve. The total antioxidant capacity of samples was determined by the FRAP assay. FRAP assay (Oyaizu, 1986) was a measurement in reduction of $Fe^{3+}(CN^-)_6$ to $Fe^{2+}(CN^-)_6$, resulting in formation of the Perl's Prussian blue complex following the addition of excess ferric ions (Fe^{3+}). One ml of berry extract (20 µg mL^{-1}) was mixed with 2.5 mL of 0.2 M (pH 6.6) phosphate buffer and 2.5 mL of 1% $K_3Fe(CN_6)$. The mixture was incubated at 50°C for 20 min, then rapidly cooled and mixed with 2.5 mL of 10% TCA then centrifuged at 1,500 rpm for 15 min. An aliquot (2.5 mL) of the supernatant was diluted with distilled water (2.5 mL) and then freshly prepared 0.5 ml of 0.1% $FeCl_3$ was added and allowed to stand for 10 min. The absorbance was measured at 700 nm. BHT (20-250 mg/mL^{-1}) was used as standard for construction of the calibration curve ($y = 0.0029\ x + 0.1542$, $R^2 = 0.99$) and reducing power was reported as BHT equivalents per mg mL^{-1} of extract.

Statistical analyses

The data was subjected to SPSS to assess the analysis of variance (ANOVA) between the quality characteristics of the accessions. The difference for the fruit characteristics of the accessions was tested by a multiple mean comparison test (Tukey) at $p \leq 0.05$ level (IBM, 2017).

Results

Morphological characterization of grape berries and clusters

Morphological characterization of the grape accessions of berry and cluster characteristics is presented in Table 1. Berry skin colour varied from red and rose to green yellow, and berry shapes ranged from ellipsoid (Kızıl Üzüm), round (Nuniyar) to ovoid. Most of the berry weight and length of the accessions were characterized as 'medium', flavours of the berries categorized as 'distinct varietal' and 'neutral' flavour. Cluster characteristics of the accessions were investigated in four groups including shape, width, and density and harvesting time of the accessions. Half of the cluster shapes of the accessions were conical, the remaining were cylindrical shape. For cluster width, 'Karaşabı' was evaluated as 'wide', whereas all the other accessions were as 'medium' or 'narrow'. Berry density on the clusters of 'Kavak Yaprağı' and 'Nuniyar' were 'loose' but 'Cevşen' and 'Dimrit' were 'dense'. 'Kırmızışabı', 'Morşabı' and 'Cevşen' were characterized as 'late' maturing and other accessions as 'medium' maturing.

Table 1. Key morphological characteristics of the grape berries and clusters based on OIV descriptors

Name	Berry characteristics					Cluster characteristics			
	Colour OIV 225	Shape OIV 223	Width OIV 221	Length OIV 220	Flavour OIV 236	Shape OIV 208	Width OIV 203	Density OIV 204	Maturity OIV 304
'Sergi Karası'	Dark Red	Ovoid	Medium	Medium	Distinct Varietal	Conical	Medium	Medium	Medium
'Karaşabı'	Dark Red	Ovoid	Medium	Long	Neutral	Conical	Wide	Medium	Medium
'Kırmızışabı'	Dark Red	Ovoid	Medium	Medium	Neutral	Cylindrical	Narrow	Loose	Late
'Morşabı'	Rose	Ovoid	Medium	Long	Neutral	Cylindrical	Medium	Dense	Late
'Nuniyar'	Rose	Round	Narrow	Short	Distinct Varietal	Conical	Medium	Loose	Medium
'Kızıl Üzüm'	Rose	Ellipsoid	Medium	Medium	Neutral	Conical	Medium	Medium	Medium
'Tilki Kuyruğu'	Green Yellow	Ovoid	Narrow	Medium	Neutral	Cylindrical	Narrow	Medium	Medium
'Cevşen'	Green Yellow	Ovoid	Narrow	Medium	Neutral	Conical	Narrow	Dense	Late
'Kavak Yaprağı'	Green Yellow	Ovoid	Medium	Medium	Distinct Varietal	Conical	Medium	Loose	Medium
'Dimrit'	Green Yellow	Ovoid	Medium	Medium	Neutral	Cylindrical	Narrow	Dense	Medium

Physical measurements on the grape clusters and berries

Physical parameters included cluster weight, 100-berry weight, must yield, berry skin firmness, berry shape index, and chroma index of the grapes are presented in Table 2. The cluster weights of the grape accessions ranged from 254 g for 'Sergi Karası' to 528 g for 'Dimrit' with an average of 352 g. The difference in the cluster weights of the 'Dimrit', 'Kavak Yaprağı' and 'Sergi Karası' were significant. However, significant differences in cluster weight were not found between the other grape accessions. According to the OIV 502, clusters of 'Sergi Karası' and 'Kavak Yaprağı' were classified as 'low' and 'medium' in cluster weight, respectively. The other accessions were positioned between them. The 100-berry weight of accessions varied significantly from each other. The heaviest was 'Kavak Yaprağı' at 452 g followed by 'Morşabı', 'Kırmızışabı' and 'Karaşabı', all having above the average berry weight. 'Sergi Karası', 'Kızıl Üzüm' and 'Nuniyar' the lowest berry weight. The volume of berry must yield is given in Table 2. The must yield of the berries ranged from 62% for 'Cevşen' to 82% for 'Kavak Yaprağı' with the average of 69%. The highest must yield for 'Kavak Yaprağı' was followed by 'Nuniyar', 'Kızıl Üzüm', and 'Sergi Karası' with 72.2, 72.0, and 70.1%, respectively. For OIV 233, all grape accessions were scored as 'medium' for must yield, except for 'Kavak Yaprağı', which was classified as 'high'. The higher firmness values were with 'Kızıl Üzüm', 'Nuniyar' and 'Tilki Kuyruğu' (4.7, 4.1 and 3.8 N) compared to other accessions. Berry shape was estimated as the ratio of width to length to determine. This parameter is especially important for selection of table or raisin grapes. 'Nuniyar' was classed as round but the other accessions ranged from ovoid to ellipsoidal. The differences in chroma index of grape accessions were significant and varied from 3.2 for for 'Nuniyar' to 6.6 'Karaşabı' with the average chroma index value of 4.5. Highest chroma index was measured for Tilki Kuyruğu and Karaşabı at 6.6 followed by 'Cevşen', 'Dimrit' and 'Kavak Yaprağı' at 6.0, 5.4, and 4.8, respectively.

Chemical analyses and secondary content of the accessions

The chemical parameters of the accessions including pH, TA, TSS, and TAC, TP, TF are presented in Table 3. The pH of berry juices ranged from 3.32 for 'Dimrit' to 3.52 for 'Kırmızışabı' with an average of 3.40. The pH values of the berry juices of the red accessions were higher than those of the white accessions. Also, TA varied from 0.46 for 'Kavak Yaprağı' to 0.67 for 'Tilki Kuyruğu'. Parallel with pH results, titratable acidity of the berry juices of the red accessions were clearly higher than those of the white grapes. TSS content of grape berry juice varied between 24.3 to 14.1 °Brix. 'Sergi Karası' had the highest sugar content followed by 'Nuniyar'.

Table 2. Physical quality characteristics of the accessions

	Cluster weight (g)	100-Berry weight (g)	Must yield (ml kg^{-1})	Firmness (N)	Berry shape index (width/length)	Croma index
'Sergi Karası'	254 ± 3.2c	213±5.4e	701±10.5c	2.4±0.8e	0.95±0.05c	1.3±0.1g
'Karaşabı'	366 ± 26.7b	371±8.9c	680±11.8cd	3.2±0.1c	0.84±0.006e	6.6±0.4a
'Kırmızışabı'	358 ± 16.6b	382±14.2bc	648±13.1e	2.8±0.1d	0.82±0.004e	1.8±0.1f
'Morşabı'	325 ± 7.9bc	402±12.9b	650±13.7e	2.8±0.2cd	0.83±0.06e	3.6±0.2e
'Nuniyar'	333 ± 30.7bc	238±4.0e	722±12.4b	4.1±0.1b	1.00±0.02a	3.2±0.1e
'Kızıl Üzüm'	325 ± 7.9bc	223±11.2e	720±10.2b	4.7±0.1a	0.78±0.01f	5.1±0.1cd
'Tilki Kuyruğu'	372 ± 38.2b	294±4.6d	675±10.8d	3.8±0.1b	0.97±0.06bc	6.6±0.1a
'Cevşen'	298 ± 15.5bc	295±12.3d	620±12.1f	3.2±0.1c	0.95±0.01c	6.0±0.1b
'Kavak Yaprağı'	372 ± 29.4b	452±3.6a	820±12.2a	2.0±0.1e	0.98±0.01ab	4.8±0.1d
'Dimrit'	528 ± 56.3a	286±6.9d	670±10.2d	2.3±0.1e	0.89±0.006d	5.4±0.1c
Average	**352**	**316**	**690.50**	**3.12**	**316**	**4.5**

The data are expressed as means ± SD with the same letters in the same column are not significantly different at p≤0.05 level.

Table 3. Chemical quality characteristics and secondary metabolite contents of berry ethanolic extracts of the grape accessions

Accessions	**pH**	**TA** (% of tartaric acid)	**TSS** (°Brix)	**TSS/ TA**	**TAC** (mg malvidin-3-glucoside kg^{-1} FW)	**TP** (mg GAE kg^{-1} FW)	**TF** (mg CE kg^{-1} FW)
'Sergi Karası'	3.39 ± .02cd	0.64 ± .02ab	24.3±0.7a	38.02 ± 0.8b	319.6±11.7bc	3,036.9±178.1a	1,296.9±40.9a
'Karaşabı'	3.42 ± .01bc	0.50 ± .01cd	19.1±0.2c	37.59 ± 0.9b	558.4±46.7a	2,224.3±149.6b	806.6±57.4de
'Kırmızışabı'	3.52 ± .02a	0.54 ± .02c	19.0±0.7c	35.00 ± 0.5c	623.3±48.9a	1,492.4±122.7cd	648.1±27.6e
'Morşabı'	3.45 ± .01b	0.60 ± .01b	18.7±0.9c	31.03 ± 1.2d	102.2±17.6e	870.2±35.4e	652.2±24.9e
'Nuniyar'	3.41 ± .01bc	0.60 ± .01b	22.4±0.2b	37.16 ± 1.0b	291.4±6.7bc	2,439.8±92.5b	995.5±66.1bc
'Kızıl Üzüm'	3.25 ± .01f	0.59 ± .01b	19.2±0.5c	32.52 ± 1.1d	329.2±11.1bc	1,552.4±77.9cd	987.7±31.2bc
'Tilki Kuyruğu'	3.28 ± .01f	0.67 ± .01a	14.1±0.4d	20.81 ± 1.3e	213.8±5.7d	2,758.4±98.2a	1,064.5±36.4b
'Cevşen'	3.44 ± .01bc	0.47 ± .01de	18.9±0.2c	39.42 ± 0.5a	351.3±21.9b	1,755.4±56.0c	1,089.0±58.5b
'Kavak Yaprağı'	3.37 ± .02d	0.46 ± .02e	18.4±0.3c	40.35 ± 0.5a	206.8±9.5d	1,247.3±54.9d	755.7±46.8de
'Dimrit'	3.32±.02e	0.61 ± .02b	18.2±0.5c	29.68 ± 1.5d	259.7±4.4cd	1,450.9±40.7cd	860.4±83.0cd
Average	**3.40**	**0.57**	**19.22**	**34.16**	**325.0**	**1882.8**	**915.6**

The data are expressed as means ± SD with the same letters in the same column are not significantly different at p≤0.05 level.

The TSS content of all the others was similar to each other because grapes were harvested at commercial maturation °Brix level. The ratio of °Brix to acidity can give a better measurement of palatability of grapes to consumers than either the sugar content or acidity alone. TSS/TA ratio varied form 20.8 for 'Tilki Kuyruğu' to 40.4 for 'Kavak Yaprağı'. TAC content of grape berries ranged from 102 for 'Morşabı' to 623.3 mg malvidin-3-glucoside kg^{-1} FW for 'Kırmızışabı'. TAC content varied with berry skin colour; red grape accessions, 'Kırmızışabı' and 'Karaşabı', had the highest TA contents, followed by rose colour vine grape berries, 'Nuniyar' and 'Kızıl Üzüm', and the lowest TAC was measured in white grape berries, 'Tilki Kuyruğu' and 'Kavak Yaprağı'. In contrast to TAC content, the second highest TP was obtained from one white grape accession 'Tilki Kuyruğu' but the highest TP was in red grape 'Sergi Karası' followed by 'Nuniyar' and 'Karaşabı' accessions. Similar to TAC content, the lowest TP was in 'Morşabı' then, 'Kavak yaprağı'. Finally, the highest and lowest TF were obtained from the grape accessions with red skin colour, 'Sergi Karası' and 'Kırmızışabı' with 1,297 and 648 mg CE kg^{-1} FW, respectively. 'Cevşen' and 'Tilki Kuyruğu' were relatively high with 1,089 and 1,064 mg CE kg^{-1} FW of TF, respectively, and then followed by the other accessions 'Kızıl Üzüm', 'Nuniyar' and 'Dimrit' accession in that order (Table 3).

Antioxidant potential of grape berry extracts

Antioxidant activity of the accessions measured by DPPH assay is show in Figure 3. DPPH inhibition activities of the grape accessions were distinctly different each other at different extract concentrations. The highest DPPH activity was measured in t 'Tilki Kuyruğu' at all extract concentrations and the lowest in 'Mor Şabı' followed by 'Kavak Yaprağı'. DPPH inhibition of all other grape extracts were similar to each other (Figure 3). There was an inverse relationship between antioxidant activity and IC_{50} across the accessions (Table 4). The FRAP values ranged between accessions from 117 µg BHT mL^{-1} ethanolic fruit extract for 'Sergi Karası' to 22.5 for Kavak Yaprağı followed by 'Tilki Kuyruğu', 'Cevşen' and 'Kızıl Üzüm' (Table 4). The average FRAP value of the samples was 51.3 µg BHT mL^{-1} of extract, the lowest FRAP capacity was in 'Kavak Yaprağı' at 22.5 µg BHT mL^{-1} of extract, followed by 'Karaşabi' and 'Kırmızışabı' accession.

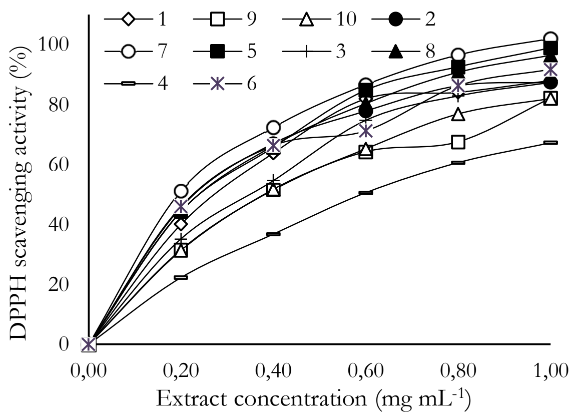

Figure 3. DPPH free radical scavenging activity of ethanolic berry extracts of the accessions

Table 4. IC50 and FRAP activity extracts of the accessions

	IC_{50} (mg mL^{-1})	FRAP (µg BHT mL^{-1})
'Sergi Karası'	0.28±0.01a	117.2±2.8a
'Karaşabı'	0.28±0.02a	36.1±4.7de
'Kırmızışabı'	0.30±0.04a	36.4±4.6de
'Morşabı'	0.59±0.02c	5.9±1.8f
'Nuniyar'	0.28±0.04a	74.0±4.1bc
'Kızıl Üzüm'	0.29±0.01a	45.1±4.0d
'Tilki Kuyruğu'	0.24±0.01a	81.5±5.0b
'Cevşen'	0.28±0.01a	61.0±3.3c
'Kavak Yaprağı'	0.42±0.01b	22.5±3.4e
'Dimrit'	0.29±0.01a	33.0±9.6de
Average	**0.34**	**51.30**

Values are expressed as means ± SD with the same letters in the same column are not significantly different at $p \leq 0.05$ level

FRAP capacity of the grape accessions were significantly different each other and varied from 117.2 'Sergi Karası' to 22.5 µg BHT mL^{-1} ethanolic fruit extract of 'Kavak Yaprağı' aceesion followed by 'Tilki Kuyruğu', 'Cevşen' and 'Kızıl Üzüm' (Table 4). While the average FRAP value of the samples was 51.3 µg BHT mL^{-1} of extract, the lowest FRAP capacity between the grape accessions was 'Kavak Yaprağı' with 22.5 µg BHT mL^{-1} of extract, followed by 'Karaşabi' and 'Kırmızışabı' accession.

Discussion

This study provided information on fruit quality parameters including morphological, physical, chemical, and antioxidant potential of the grapevine accessions from heritage germplasm grown in Central Anatolia. Fruit skin colour is one of the most important phenotypic characters of the table grape cultivars, and is of high importance for consumer preference (Somogyi *et al*., 2020). Research has also indicated that primary quality parameters for the table grape is not only colour and berry size, but also the cluster size and shape are important (Harindra Champa, 2015; Cliff *et al*., 1996). Based on the literature, bright berry skin colour, large berry size and unusual cluster shape might be attractive for the consumer due to its rarity. In the study, berry uniformity in the cluster and cluster morphological characteristics for the majority of the accessions met table quality criteria and might be accepted as fresh table grapes, the exceptions were 'Gevşen' and 'Dimrit'. These two accessions might be crushed and used to make wine, or fresh fruits juice. 'Sergi Karası' could also be promising genotype for the raisin due to its distinctive traits with small berries and dense cluster (Table 1).

Each accession had its own physical characteristics (Table 2) that might suit them for specific uses and directly effective on storage time and shelf life (Rolle *et al*., 2012). The results showed that grape genotypes, environmental conditions and cultural practices were suitable in terms of cluster weight, which ranged from 100 and 350 g, although some hybrids grapes can have much heavier clusters. A good quality table grape is defined as medium sized clusters with perfect berries, characteristic colour, acceptable flavour, and the texture (Winkler *et al.*, 1974). Similarly, berry size is always an important quality factor for winemaking, with medium berries making more consistent wines and scoring higher than other sizes (Melo *et al*., 2015). Must ratio and firmness of the accessions be the crucial parameters determining eating quality of grapes and suitability for their handling and long-term storage (Fuentes *et al.*, 2018; Maltini *et al*., 2003). The physical characteristics of 'Nuniyar', 'Kızıl Üzüm' and 'Tilki Kuyruğu' might suit them for table grapes, based on information in the literature, and cold storage. 'Kavak Yaprağı' could be suitable as fresh table grape without cold storage.

The quality of table grapes with sufficient sugar, acidity, aroma, flavour and nutraceutical traits is determined by genetic and environmental factors. pH is important factor for the biological stability of grape juice and wine as well as having an effect on the ionic forms of some organic molecules, such as anthocyanins, affecting their colour. Keller (2010) reported that a pH higher than 3.6 is usually undesirable as it caused a low intensity of colour, impairs microbial stability, increased susceptibility to oxidation and raised the spoilage potential of the wine produced. The pH of all the accessions in the presented study ranged from 3.25 to 3.52 (Table 3) which was ideal for table grapes, drying or winemaking. Although the best measure of quality varies between grape genotype, the ratio of TSS/TA can provide a more reliable indicator of the palatability of grapes to consumers than either the sugar content or acidity alone (Winkler *et al.*, 1974). Consistent with the literature, 'Kavak Yaprağı' and 'Cevşen' had highest TSS/TA ratio giving them excellent tastes as table grape followed by 'Nuniyar', 'Karaşabı' and 'Kırmızışabı'. The sugar-acid ratio had a great effect on the taste of the accessions. The accessions had good sensory qualities when they were harvested at a °Brix >15. Also, 'Sergi Karası' had the highest TSS (24.3 °Brix) making it suitable to it raisin production (Table 3).

Recently the phytochemical attributes of grape cultivars have been given greater consideration and cultivars can be grouped according to their distinctive secondary metabolites (Moreno *et al*., 2015). Anthocyanins, phenolics and flavonoids, and consequent antioxidant potential, were assessed in grape berries of heritage accessions in the study. Anthocyanins are pigments being end-products of the flavonoid pathways, which contribute to the majority of red colour to fruit, juice and wine (Liang *et al*., 2008). Flavonoids are responded for bitterness and aroma (Adams, 2006) and phenolics, found in all vine tissues, are also in juice or wine so can alter the character of the resulting product with their content in grapes varying according to genotype and environmental conditions (Creasy and Creasy, 2009). The highest anthocyanins were measured in red grape accessions 'Kırmızışabı', 'Karaşabı' and 'Sergi Karası' followed by rose accessions 'Kızıl Üzüm' and

'Nuniyar', then white accessions 'Kavak Yaprağı' and 'Dimrit' accessions. Berry skin colour of accessions was a sole determinant on the anthocyanin content of berries (Table 3).

In contrast to anthocyanin content, 'Tilki Kuyruğu', a white accession, had the second highest phenolic content after 'Sergi Karası' with red berries. This result was consistent with the study of Ozden and Vardin (2009) showing that grape cv. Chardonnay the higher total phenolics compared with Merlot, Syrah and Cabernet Sauvignon grown under the same conditions. Based on this finding, total phenolic contents of accessions grown under similar conditions varied significantly each other due to genotypic differences (Table 3). Total flavonoid content of accessions showed a similar trend to phenolic content. Regardless of berry skin colour, total flavonoid content of the accessions varied and the highest flavonoids were orderly measured from the berries of 'Sergi Karası', 'Cevşen' and 'Tilki Kuyruğu'. Recently, it has been recognised that the secondary metabolite profile and antioxidant potential of grapes can be used for discriminating genotypic and geographic of origin using the chemical nature and behavior of polyphenols (Maletic *et al.*, 2009; Bindon *et al.*, 2013; Laurentiu and Popa, 2018).

The DPPH free radical scavenging activity of ethanolic fruit juice extracts was highest in 'Tilki Kuyruğu' due to high phenolic and flavonoid content. In contrast contrary, 'Morşabı' had the lowest DPPH free radical scavenging activity with the lowest secondary metabolite profiles analyzed in the study. The IC_{50} values of the accessions were calculated by using polynomial equations of DPPH scavenging activities of grapes. The lowest IC_{50} value was obtained from extracts of 'Tilki Kuyruğu' (0.24 mg mL^{-1}), and the highest from 'Morşabı' (0.59 mg mL^{-1}). In the present study, the average of IC_{50} value was 0.34 mg mL^{-1}. FRAP reducing power of extracts showed similar trend to DPPH free radical activity, and highest FRAP reducing power was with 'Sergi Karası' and 'Tilki Kuyruğu' followed by 'Nuniyar' and 'Cevşen'. Based on these findings, the accessions with rich secondary metabolite profiles contributed to inhibit DPPH free radicals and to increase FRAP ion reducing power of extracts. These results are consistent with some studies that showed higher secondary metabolite content reflected higher antioxidant power of plant samples (Wu *et al.*, 2006; Cui *et al.*, 2010; Lucia *et al.*, 2020). Based on these data, the results make it possible to suggest that secondary metabolite content and antioxidant power of grapes might be used as a biochemical marker to discriminate grape genotypes. Also knowing grape morphological, physical and chemical characteristics including phenolics, anthocyanins and flavonoids, as well as the antioxidant power of grapes might be of benefit in introducing new cultivars and increasing their consumption. The variabilities in fruit quality characteristics of the accession tested indicate that this is valuable heritage germplasm that could be considered for preservation and certification.

Conclusions

This study showed valuable and practical data about morphological, physical and chemical content of some grape accessions from heritage germplasm grown in Central Anatolia, Turkey. 'Karaşabı,' 'Kırmızışabı', 'Nuniyar', 'Kızıl Üzüm', and 'Kavak Yaprağı' accessions met the quality characteristics of table grapes, 'Sergi Karası' could be used as raisin, and 'Cevşen' and 'Dimrit' for wine making. Also all grape accessions had remarkable antioxidant power, strong relationship between secondary metabolite content and antioxidant activity was found. Since the region where this study was carried out is very rich in grape diversity, it should be screened and accessions protected by the project supported by institutions or organizations.

Authors' Contributions

MO guided the study, collected the samples, analyzed the data and wrote the manuscript; YD performed the laboratory work and analyzed the data. Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Adams DO (2006). Phenolics and ripening in grape berries. American Journal of Enology and Viticulture 57:249-256. *https://doi.org/10.5344/ajev.2006.57.3.249*

Aday MS, Caner C (2010). Understanding the effects of various edible coatings on the storability of fresh cherry. Packaging Technology and Science 23(8):441-456. *https://doi.org/10.1002/pts.910*

Anonymous (2009). OIV Technical Standards and Documents. Code des caracteres descriptifis des varietes et especes de vitis (OIV) Paris, 2009. Retrieved 2021 February 20th from: *https://www.oiv.int/public/medias/2274/code-2e-edition-finale.pdf*

Bindon K, Varela C, Kennedy J, Holt H, Herderich M (2013). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. Food Chemistry 138:1696-1705. *https://doi.org/10.1016/j.foodchem.2012.09.146*

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. Nature 181:1199-1200. *https://doi.org/10.1038/1811199a0*

Cliff MA, Dever MC, Reynolds AG (1996). Descriptive profiling of new and commercial British Columbia table grape cultivars. American Journal of Enology and Viticulture 47(3):301-308.

Creasy GL and Creasy LL (2009). Grapes. Cambridge: CAB International.

Cui SH, Murthy HN, Wu CH, Paek KY (2010). Sucrose-induced osmotic stress affects biomass, metabolite, and antioxidant levels in root suspension cultures of *Hypericum perforatum* L. Plant Cell Tissue and Organ Culture 103:7-14. *https://doi.org/10.1007/s11240-010-9747-z*

Dvorakova M, Landa P (2017). Anti-inflammatory activity of natural stilbenoids: A review. Pharmacological Research 124:126-145. *https://doi.org/10.1016/j.phrs.2017.08.002*

Ergul A, Kazan K, Aras S, Cevik V, Celik H, Söylemezoglu G (2006). AFLP analysis of genetic variation within the two economically important Anatolian grapevine (*Vitis vinifera* L.) varietal groups. Genome 49:467-495. *https://doi.org/10.1139/g05-121*

Fuentes S, Manso, A, Fenoll J, Cava J, Garrido I, Molina MV, … Hellin, P (2018). Optimizing the methodology to measure firmness of grape berries (*Vitis vinifera* L.) during ripening. Acta Horticulturae 1194:1103-1110. *https://doi.org/10.17660/ActaHortic.2018.1194.158*

Giusti MM, Wrolstad RE (2003) Acylated anthocyanins from edible sources and their applications in food systems. Biochemical Engineering Journal 14:217-225. *https://doi.org/10.1016/S1369-703X(02)00221-8*

Gu S, Ding P, Howard SY (2002). Effect of temperature and exposure time on cold hardiness of primary buds during the dormant season in 'Concord', 'Norton', 'Vignoles' and 'St. Vincent' grapevines. Journal of Horticultural Science and Biotechnology 77(5):635-639. *https://doi.org/10.1080/14620316.2002.11511550*

Harindra Champa WA (2015). Pre and postharvest practices for quality improvement of table grapes (*Vitis vinifera* L.). Journal of the National Science Foundation of Sri Lanka 43(1):3-9. *http://dx.doi.org/10.4038/jnsfsr.v43i1.7921*

Hwang D, Lim YH (2019). Resveratrol controls *Escherichia* coli growth by inhibiting the AcrAB-TolC efflux pump. FEMS Microbiology Letters (1):366(4):fnz030. *https://doi.org/10.1093/femsle/fnz030*

IBM (2017). IBM Analytics. *https://www.ibm.com/analytics/spssstatistics-software*

Keller M (2010). The Science of Grapevines - Anatomy and Physiology. In Elsevier: Academic Press, Burlington, 2010.

Laurentiu MP, Popa ME (2018). Polyphenol fingerprinting approaches in wine traceability and authenticity: Assessment and implications of red wines. Beverages 4:75-92. *https://doi.org/10.3390/beverages4040075*

Leifert WR, Abeywardena MY (2008). Cardioprotective actions of grape polyphenols. Nutrition Research 28:729-737. *https://doi.org/10.1016/j.nutres.2008.08.007*

Liang Z, Wu B, Fan P, Yang C, Duan W, Zheng X, …Li S (2008). Anthocyanin composition and content in grape berry skin in *Vitis* germplasm. Food Chemistry 111:837-844. *https://doi.org/10.1016/j.foodchem.2008.04.069.*

Lucia P, Moccia F, Nasti R, Marzorati S, Verotta L, Napolitano A (2020). Bioactive phenolic compounds from agri-food wastes: An update on green and sustainable extraction methodologies. Frontiers in Nutrition 7:60. *https://doi.org/10.3389/fnut.2020.00060*

Maletic E, Kontic JK, Preiner D, Jeromel A, Patz CD, Dietrich H (2009). Anthocyanin profile and antioxidative capacity of some autochthonous Croatian red wines. Journal of Food, Agriculture and Environment 7:48-51. *https://doi.org/10.1234/4.2009.1404*

Maltini E, Torreggiani D, Venir E, Bertolo G (2003). Water activity and the preservation of plant foods. Food Chemistry 82:79-86. *https://doi.org/10.1016/S0308-8146(02)00581-2*

Manach C, Scalbert A, Morand C, Remesy C, Jimenez L (2004). Polyphenols: food sources and bioavailability. American Society for Clinical Nutrition 79:727–747. *https://doi.org/10.1093/ajcn/79.5.727*

Margaryan K, Melyan G, Vardanyan D, Devejyan H, Aroutiounian R (2017). Phenolic content and antioxidant activity of Armenian cultivated and wild grapes. Bio Web of Conferences 9:02029. *https://doi.org/10.1051/bioconf/20170902029*

McGovern PE (2003). Ancient Wine: The Search for the Origins of Viniculture. Princeton, New Jersey. Princeton University Press.

McGuire R (1992). Reporting of objective color measurements. American Society for Horticultural Science 27:1254-1255. *https://doi.org/10.21273/hortsci.27.12.1254*

Melo MS, Schultz HR, Volschenk CG, Hunter JJ (2015). Berry size variation of *Vitis vinifera* L. cv. Syrah: Morphological dimensions, berry composition and wine quality. South African Journal for Enology and Viticulture 36(1):1-10. *https://doi.org/10.21548/36-1-931*

Moreno MM, Olalla HM, Gimenez MR, Navarro AlM, Rufian HJA (2015). Phenolic compounds and antioxidant activity of Spanish commercial grape juices. Journal of Food Composition and Analysis (38):19-26. *https://doi.org/10.1016/j.jfca.2014.10.001*

Nivelle L, Hubert J, Courot E, Jeandet P, Aziz A, Nuzillard JM, … Delmas D (2017) Anti-cancer activity of resveratrol and derivatives produced by grapevine cell suspensions in a 14 L stirred bioreactor. Molecules 22(3):474. *https://doi.org/10.3390/molecules22030474*

Oyaizu, M (1986) Studies on product of browning reaction prepared from glucose amine. Japan Journal of Nutrition 44:307-315. *http://dx.doi.org/10.5264/eiyogakuzashi.44.307*

Ozden M and Vardin H (2009). Quality and phytochemical properties of some grapevine cultivars grown in Şanliurfa conditions. Journal of Harran Agriculture and Food Science 13(2):21-27.

Parry J, Su L, Moore J, Cheng Z, Luther M, Rao JN, Wang JY, Yu LL (2006). Chemical compositions, antioxidant capacities, and antiproliferative activities of selected fruit seed flours. Journal of Agricultural and Food Chemistry 54(11):3773-3778. *https://doi.org/10.1021/jf060325k*

Rankine, BC, Cellier KM, Boehm EW (1962). Studies on grape variability and field sampling. American Journal of Enology and Viticulture 13(2):58-72.

Razvan VF, Roxana MF, Ancuta N, Marius MB, Liliana R, Cristina A, Antoanela P (2017). Assessment of quality characteristics of new *Vitis vinifera* L. cultivars for temperate climate vineyards. Acta Agriculturae Scandinavica, Section B — Soil and Plant Science 67(5):405 415. *https://doi.org/10.1080/09064710.2017.1285959*

Rolle L, Siret R, Rio SS, Maury C, Gerbi V, Jourjon F (2012). Instrumental texture analysis parameters as markers of tablegrape and winegrape quality: A review. American Journal of Enology and Viticulture 63:11-28. https://doi.org/10.5344/ajev.2011.11059

Slinkard K, Singleton VL (1977). Total phenol analysis: automation and comparison with manual methods. American Journal of Enology and Viticulture 28:49-55.

Somogyi E, Lazar J, Bodor P, Kaszab T (2020). Colour of grapevine (*Vitis vinifera* L.) accessions influenced by the length of cold storage grapevine berry colour measurement. Progress in Agricultural Engineering Sciences 16:109-116. https://doi.org/10.1556/446.2020.20013

Tako M, Kerekes EB, Zambrano C, Kotogan A, Papp T, Krisch J, Vagvolgyi C (2020). Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. Antioxidants 18(2):165. https://doi.org/10.3390/antiox9020165

Vavilov NI (1926). Studies on the Origin of Cultivated Plants. Leningrad. Institute of Applied Botanical Plant Breeding.

Winkler AJ, Cook JA, Kliewer WM, Lider LA (1974). General Viticulture. 2nd Edition, University of California Press, pp 710, Berkeley

Wrolstad RE (1976). Color and pigment analyses in fruit products. In: Agricultural Station Bulletin 624, Oregon State University.

Wu CH, Dewir YH, Hahn EJ, Paek KY (2006). Optimization of culturing conditions for the production of biomass and phenolics from adventitious roots of *Echinacea angustifolia*. Journal of Plant Biology 49:193-199. https://doi.org/10.1007/BF03030532

Yılmaz F, Shidfar M, Hazrati N, Kazan K, Ozmen CY, Uysal T, … Ergul A (2020). Genetic analysis of central Anatolian grapevine (*Vitis vinifera* L.) germplasm by simple sequence repeats. Tree Genetics and Genomes 16:55. https://doi.org/10.1007/s11295-020-01429-z

Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64:555-559. https://doi.org/10.1016/S0308-8146(98)00102-2