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POMOLOGICAL AND CHEMICAL CHARACTERISTICS OF APPLE GENOTYPES IN SIMAV DISTRICT, KUTAHYA, TURKEY

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Abstract

This study was carried out to determine the pomological (physical) and chemical characteristics of some local apple genotypes grown in Simav district of Kutahya, Turkey. Due to the negative effects of climate change, the adaptability of local genotypes to the environmental stress conditions is becoming an important tool for reaching sustainability in production. However, it this case, the pomological and chemical characteristics of the local genotypes is becoming an important question for the consumers and have to be answered before the cultivation and marketing of the local genotypes. As a result of the surveys conducted in Simav district, six different apple genotypes were collected for analysis. According to the results obtained, the highest fruit weight was found in SIM-04 genotype as 205.34 g; and the lowest fruit weight was in the SIM-06 as 130.51 g. We found values of total phenols between 348.13 mg GAE/l (SIM-01) and 265.49 mg GAE/l (SIM-05). Total antioxidant activity was determined between 7.28 % (SIM-01) and 4.13 % (SIM-02). Total flavonoids were measured between 732.11 GAE/l (SIM-03) and 400.37 GAE/l (SIM-06). Malic acid is the most important acid in apple and genotypes malic acid content ranges between 3526.70 mg/100 ml (SIM-03) and 2448.51 mg/100 ml (SIM-06). Results suggested that there is a significant difference between the pomological and chemical characteristics of the genotypes and the selection of the most appropriate genotype is highly dependent upon the preferred characteristics of the apples. It can be recommended from the results that the SIM-04 is superior in terms of pomological characteristics. Moreover, when the preferred characteristics are the antioxidant activity and total phenols, SIM-01 genotype is superior.

Key words: Antioxidant activity, Total phenols, Total flavonoids, Malic acid, Oxalic acid.

Introduction

Apple is a species of the genus *Malus* belonging to the *Rosaceae* family (Unal, 2011). The homeland of apple is the Southern Caucasus, which includes Anatolia. Due to favorable climatic conditions, Turkey is one of the countries with a significant share of production (4^{th} place with 4.21% of production in 2018) in the world's apple production (Anon., 2020).Apple cultivation of many varieties (i.e. Starking Delicious' and 'Golden Delicious) is maintained in almost every region of Turkey (Mordoğan & Ergun, 2002; Giray *et al.,* 2019). More than half of the world's apple production is maintained in the Asian continent. The temperate climatic zone in Asia provides favorable conditions for growing apples and is an important center for germplasm. Although the America contitent has lower share of production as compared to Asia and Europe, the continent ranks third in apple production (Öztürk *et al*., 2011; Lipa *et al.,* 2019; Szot & Lipa, 2019). Apple is one of the most flexible fruit species in terms of climate and soil requirements due to variety and rootstock richness suitable for different ecology and soil structure (Hampson & Kemp, 2003; Đorđević *et al.,* 2019).

Reactive oxygen species (ROS) are well-known for causing destruction in the cells and being the main reason for many health related problems (Bardaweel *et al*., 2018). It is also known that the ROS accumulation in human body increases with the age of human. Therefore, it is important to minimize free radical production by life modification and have maximum availability of antioxidants for a healthier life. These changes include smoking cessation, minimization of sun exposure, prevention of air pollution and non-inhalation of toxic chemicals such as exhaust fumes, and reduction of alcohol intake. Fruits, cereals, legumes and vegetables are rich antioxidants known as phytochemical elements that function as true natural drugs (Gemma *et al*., 2007; Butiuc *et al.,* 2019). Antioxidants are the compounds which prevent the formation of free radicals and they operate to minimize the damage of free radical formation. The term antioxidant is applied to the compounds because of their characteristics to fight with oxidation. Antioxidants are substances that react with body chemicals, free radicals and other reactive oxygen species to prevent the oxidation process. Antioxidants close the free radical gap by giving electrons without destabilizing themselves to stop the free radical chain reaction (Szalay, 2016). Substances such as ascorbic acid (vitamin C), bilirubin and glutathione peroxidase (selenium), uric acid, superoxide dismutase (such as copper, zinc, manganese, glutathione, tocopherol (vitamin E), catalase (iron), β-carotene, and many metalloenzymes protect the body against free radical damage (Machlin & Bendich, 1987; Tkaczuk *et al.,* 2019).

Therefore, phenolic acids, natural antioxidant, and derivatives present in the diet or synthetically prepared have promising chemo preventive properties which are identified as useful agents for future development (Fang *et al*., 2002; Barreira *et al.,* 2008). Fruits are known to have positive effects on human health, especially due to the presence of phenolic acids that they contain. Phenolic acids has been reported to be effective in preventing lung cancer, colon and prostate cancer (Vermerris & Nicholson, 2006; Eskimez *et al.,* 2019; Gündeşli *et al.,* 2019; Milosevic *et al.,* 2019; Okatan, 2020).

Apple is one of the most produced and marketed fruits in the world. It is favored by producers and consumers due to its high adaptability to different climate terms and high nutritional rates. However, the production of apples is limited in many areas, due to the climate change and reduction in the available ecological resources. Thus, there is a need for different apple genotypes suitable for various ecological conditions. In our study, the domestic apple genotypes grown in Simav district of Kutahya were marked and the pomological and chemical characteristics of these apples were compared. It is essential to preserve the resources of the genes, which may extinct with time, and affect the future reproduction activities.

Materials and Methods

Plant materials: Preliminary contact was carried with the growers located in the Simav district of Kütahya province. The information collected from the growers suggested that there are six different apple genotypes in the Simav district. Simav is a town of Kütahya province. It is located 143 km southwest of the city center in Central Anatolia. The different genotypes were determined according to the information collected from the growers and to the appearance of the trees and fruits. The selected trees were painted and the fruits were collected at full ripeness duration. The marked trees of native genotypes in the district were named as SIM-01, SIM-02, SIM-03, SIM-04, SIM-05, and SIM-06. Global Positioning System (GPS) coordinates and altitudes of selected apple genotypes are presented in Table 1. Collected fruit samples were brought to the Scientific Analysis Technological Application and Research Center, Uşak University.and prepared for analysis.

Table 1. GPS coordinates and altitude of apple genotypes.

Genotypes	Coordinates		Altitude (m)
	North	East	
SIM-01	39°06’11.01"	28°54’64.33"	995
SIM-02	39°05’58.40"	28°55’00.42"	882
SIM-03	39°06’06.81"	28°54’57.05"	852
SIM-04	39°08’00.17"	28°54’07.78"	788
SIM-05	39°07’44.07"	28°54’22.62"	790
SIM-06	39°06’18.13"	28°52’18.27"	900

Methods: A total of 30 fruits were collected from each genotype. Thus, the fruits were randomly divided into three groups (replication) with 10 fruits in each. The collected apple samples were preserved in a refrigerator (at +4 °C) for no more than 1 day until the analyses are completed.

Pomological analyses: The weights of the harvested fruits were measured as grams, with a digital scale (sensitivity of 0.01 g). The width and length of fruits, the thickness and length of the fruit stalks were measured by caliper (sensibility of 0.01 mm). Finally, fruit firmness was measured with a penetrometer and all data was noted for further analysis.

Chemical analyses: Total soluble solids content (TSS) of the genotypes were determined by a digital-refractometer (REF 103 / Index Instruments Ltd.). Freshly collected apple samples were squeezed by grinding in a mixer and then the juice was obtained by filtration the mixture through filter paper. Next, 50 ml of fresh juice was taken from each fruit sample to measure pH values using the pH meter (Hanna pH 211, Germany). To measure titratable acidity (TA) of the genotypes, 25 ml of freshly squeezed apple juice was placed in a 250 ml beaker. After the addition of 50 mL of distilled water, the mixture was kept in a hot water bath with continuous stirring for 30 minutes. The mixture was cooled and the contents of the beaker were filtered by using filter paper (Whatman No.1). The filtrate was diluted to 100 ml with distilled water. The contents were then transferred into a 250 ml erlenmeyer flask and titrated with 0.1 N NaOH solution in the presence of phenolphthalein indicator, till an end point pH value of 8.2. Titratable acidity content was then determined as malic acid (Campeanu *et al*., 2009).

Antioxidant activity was determined using 2,2-diphenyl-1-picryl-hydrazyl-hydrate $(DPPH)^{\cdot}$ scavenging method. The extraction and the measurement procedures were carried out following the method described by Plaza *et al*., (2016). A total of 5 mL apple juice was mixed with 5 mL of methyl alcohol (80%) and centrifuged at 4000 rpm for 10 min. Hereafter 0.1 mL of supernatant was added to 2.46 ml of DPPH and vortexed. The absorbance of the samples was measured at 515 nm using the spectrophotometer (Shimadzu UV-1800, Japan) and the percentage of DPPH scavenging activity was calculated by using the following equation:

$$\% \text{ DPPH} = [(Ac - As)/Ac] \times 100$$

where, Ac was the absorbance of the negative control (containing the extraction solvent instead of the sample) and As was the absorbance of the sample. The results were expressed as EC50 (μg/ml).The extraction and measurement of total phenolic content (TPC) was carried out by the Folin Ciocalteu method, following the changes described by Altisent *et al*., (2014). To do so, 0.2 ml of apple juice sample and 0.5 mL of Folin-Ciocalteu reagent were mixed in test tubes. Then, 1 mL of sodium carbonate (7.5% w / v) was added onto the tubes, after keeping 5 min at dark. This mixture was then kept in the dark for 1 hour. The absorbance of the solution was then read at 765 nm by UV-VIS spectrophotometer (Shimadzu UV-1800, Japan). Results were demonstrated as mg of gallic acid equivalent per 100 g of sample.

For the analysis of total flavonoid content (TFC), 0.3 mL $NaNO_2$ (5%), 0.3 mL $AlCl_3$ (10%) and 2 mL NaOH (1 M) were mixed with 5 mL of juice sample in test tubes. The contents were mixed in some quantity of distilled water, dissolved and the volume was raised to 10 mL. The absorbance was measured at 510 nm wavelength, using a UV/VIS spectrophotometer (Shimadzu UV-1800, Japan).Unit TFC content was taken as catechin equivalent/mg (Zhishen *et al*., 1999).

Organic acids of apple fruits were measured by the method of Bevilacqua & Califano (1989). Apple juice samples were put at -22°C until measurement. 5 mL of each sample was diluted with 20 mL of 0.009 NH_2SO_4 (Heidolph Silent Crusher M, Germany), then homogenized for one hour with a shaker (Heidolph Unimax 1010, Germany). The prepared mixtures were centrifuged for 15 minutes at 15,000 rpm, and the supernatants were filtered through a 0.45 μm skin strainer followed by filtration through a thick filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), and run through a SEP-PAK C18 cartridge. Organic acid measurements were performed with Agilent 1260 series HPLC (Agilent 1260 series) using an on-line degasser (G 1322A), quat pump (G 1311A), autosampler (G 1313A), column heater (G 1316A), and UV detector (G 1315A) at 214 and 280 nm wavelengths, controlled with the Agilent package program.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). All statistical analyses were performed using JMP 8 software (SAS Institute Inc., Cary, NC, USA). Normality of the data and equality of variances were tested using the Shapiro-Wilk W and Levene tests, respectively. Significant differences were calculated using one-way analysis of variance (ANOVA) and post-hoc HSD Tukey were used to check differences. The criterion for statistical significance was $p<0.05$.

Results and Discussions

Pomological characteristics of apple genotypes: Fruit weight, width and length, fruit stalk thickness and length varied in all apple genotypes at a statistically significant level of $p<0.05$ (Table 2). The highest fruit weight was found in SIM-04 (205.34 g), and the lowest in SIM-06 (130.51 g). On the other hand, the fruit widths were found to vary from 77.61 mm (SIM-04 genotype) to 55.24 mm (SIM-06). The fruit length values were found to be between 65.92 mm (SIM-01) and 54.89 mm (SIM-06). The fruit stalk thickness was determined between 2.78 mm (SIM-04) and 1.69 mm (SIMV-06). Fruit stalk length was measured between 22.44 mm (SIM-04) and 18.32 mm (SIMV-06).

Under ecological conditions of Corum (Turkey), the fruit weight of some apple varieties was found to be between 145.29 g and 209.56 g in 2010 and between 173.50 g and 205.51 g in 2009 (Çulha, 2010). In another study, Soylu *et al*., (2003) have determined the average fruit weight between 122.8 g and 169.5 g in Bursa Gorukle. The ecology of fruit weight was detected between 92.35 g and 238.50 g in Ispir (Erzurum) between 2000 and 2001 (Karlıdağ & Eşitken, 2006). Küden & Kaşka (1995) studied the pomological characteristics of apple varieties grown in Pozantı district of Adana city and they reported that the fruit weight is between 154.40 g and183.82 g. In the same study, the fruit width was noted to vary from 66.1 mm to 74.9 mm. In the ecological conditions of Samsun city, 5 old apple varieties grafted on MM106 apple rootstock were tested and the fruit stem length was determined between 21.55 mm (Jersey Mac) and 30.84 mm (Golden Delicious) (Öztürk & Öztürk, 2016). The length of the fruit stem of some apple varieties grown in Coruh valley was determined between 10.97 mm and 24.51 mm (Erdoğan & Bolat, 2002). Fruit stalk length of some apple cultivars grown in Erzurum was found to be between 11.9 mm and 24.5 mm (Kars, 2016). Results about the pomological characteristics of the six apple genotypes of present study are all found to be in agreement with the studies listed above (Küden and Kaşka, 1995; Erdoğan & Bolat, 2002; Soylu *et al*., 2003; Karlıdağ & Eşitken, 2006; Çulha, 2010; Öztürk & Öztürk, 2016; Kars, 2016; Błaszczyk & Gasparski, 2019). It is thought that differences outcome from the variations in ecological factors, differences in analysis techniques, and genetic structures of apple genotypes.

Chemical and phytochemical contents: Fruits are widely accepted as a rich source of minerals and vitamins in human nutrition. It is known that people, who consume foods rich in antioxidants, have lower incidence of various cancers and cardiovascular diseases. As a result of some scientific research, it was found that there is an inversely proportional relationship between the consumption of fruit and the occurrence of certain cancers and heart diseases (Miller & Paganga, 1996). In present study, pH, titratable acidity, TSS, TPC, antioxidant activity and TFC values were all varied in all the genotypes at a statistically significant level $p<0.05$ (Table 3). pH values were determined between 4.23% (SIM-05) and 3.13% (SIM-01). The highest titratable acidity was found as 1.19 g/L (SIM-04) and the lowest value was found 0.64 g/L (SIM-01). Total soluble solids contents (TSS) were determined between 14.16% (SIM-03) and 11.18% (SIM-03). We found values of total phenols between 348.13 mg GAE/l (SIM-01) and 265.49 mg GAE/l (SIM-05). Total antioxidant activity was determined between 7.28 % (SIM-01) and 4.13 % (SIM-02). Total flavonoids were measured between 732.11 GAE/l (SIM-03) and 400.37 GAE/l (SIM-06).

Table 2. Pomological characteristics of six apple genotypes in Simav district.

Genotypes	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Fruit stalk thickness (mm)	Fruit stalk length (mm)
SIM-01	190..20 b..	74..68 a..	65..92 a..	2..76 a ..	22..19 a ..
SIM-02	146..50 d..	67.32 b..	57..90 b..	2..41 b..	20..90 c..
SIM-03	177..33 c..	70..15 ab..	58..69 b..	2..45 b ..	21..11 b ..
SIM-04	205..34 a..	77..61 a..	65..21 a..	2..78 a ..	22..44 a ..
SIM-05	136..84 de..	52..79 cd..	56..51 bc..	2..34 bc..	19..57 d ..
SIM-06	130..51 e..	55..24 c..	54..89 c..	1..69 c ..	18..32 e..

Values followed by the same letter or letters in the same column are not significantly different at 0.05 level

Table 3. Chemical and phytochemical contents of genotypes.

Genotypes	pH	TA (g/L)	TSS %	TPC (mg GAE/l)	Total antioxidant activity (% DPPH)	TFC (GAE/l)
SIM-01	3.13 e .	0.64 e.	11.49 c .	348.13 a .	7.28 a .	482.53 d .
SIM-02	4.10 b.	1.05 b.	12.83 b.	275.24 e .	4.13 e .	700.91 b .
SIM-03	3.97 c .	0.67 e.	14.16 a.	311.64 c .	4.95 d .	400.37 e .
SIM-04	3.57 d.	1.19 a .	11.18 c.	296.38 d .	4.85 d .	525.16 c .
SIM-05	4.23 a.	0.78 d .	12.73 b .	265.49 ef .	6.16 c .	483.57 d .
SIM-06	4.07 b .	0.90 c.	13.44 ab .	337.98 b .	6.51 b .	732.11 a .

Values followed by the same letter or letters in the same column are not significantly different at 0.05 level

Table 4. Oxalic, malic, ascorbic and gallic acid contents (mg/100 ml) of genotypes.

Genotypes	Oxalic acid	Malic acid	Ascorbic acid	Gallic acid
SIM-01	432..68 d ..	2534..85 e ±..	41..16 c ..	18..07 c ..
SIM-02	452..76 c ..	2793..85 c ±..	50..76 b ..	23..09 b ..
SIM-03	486..89 b ..	3526..70 a ±..	62..87 a ..	21..08 bc ..
SIM-04	495..93 a ..	2614..15 d ±..	38..15 d ..	19..07 d ..
SIM-05	348..35e ..	2854..09 b ±..	43..17 bc ..	36..14 a..
SIM-06	421..64 de ..	2448..51 f ±..	50..47 b ..	15..06 e ..

Values followed by the same letter or letters in the same column are not significantly different at 0.05 level

In a previous study, Küden & Kaşka (1995) reported that the TSS values of apple varieties were between 12.4% and 13.2%. In another study, Seymen & Polat (2015) studied the morphological, phonological and pomological characteristics of some apple types grown in Amasya city of Turkey, and they determined that the TSS content varies from 11.9% to 14.6%. In the same study, pH values were noted to be between 3.08 and 4.24 and the titratable acidity changed from 0.22 g/L to 0.79 g/L. The findings obtained from similar studies conducted in different regions of our Turkey and our findings were generally parallel (Özkan & Celep, 1995; Seymen, 2015; Seymen & Polat, 2015).

Phenolic compounds play a very different role in plants. For example, some of them are effective in the formation of taste and odor elements of plants, while some others create unique colors of the plants for attracting pollinators or for protecting from herbivores (Tomás-Barberán & Espin, 2001). In one study, TPC was found between 18.30 mg / g GAE (Granny Smith) and 24.62 mg / g GAE (Pink Lady) (Ünüvar, 2014). Bahukhandi *et al.*, (2018) investigated phenolic contents and antioxidant capacities of three local apple varieties (Benoni, Fanny, and Rymer) grown in different parts of Uttarakhand (West Himalaya) in India. At the end of the study, they found that phenolic (0.90-7.00 mg / g GAE) and antioxidant activity (ABTS, 3-68; DPPH, 5–15; FRAP, 5–40 mmol/kg AAE) capacities varied significantly. Moretti *et al.*, (2010), in their study with the difference in temperature and CO_2 levels of fruit grown due to the stress state that they contain more bioactive components (phenol, ascorbic acid) reported. The effects of factors such as the region where the apple is grown, harvest time and climatic conditions are significantly effective on the amount of phenolic content (Delian *et al.*, 2011).

Content of organic acids: Organic acids have great significance in protecting human health and give flavor to the fruits. Some studies have shown that organic acids have made important contributions to humans in numerous features such as preventing kidney stones, strengthening the immune system, and eliminating oral diseases. In addition, organic acids have features such as dropping the risk of poisoning caused by toxic metals, beautifying and strengthening the skin (Abraham & Flechas, 1992; Penniston *et al.*, 2007). Oxalic, malic, ascorbic and gallic acid contents of genotypes in present study were found to vary significantly at $p<0.05$ (Table 4). Oxalic acid values were determined between 495.93 mg/100 ml (SIM-04) and 348.35 mg/100 ml (SIM-05). In the apple genotypes examined, malic acid was found to be largest among organic acid materials. Malic acid is the most important acid in apple and genotypes has malic acid between 3526.70 mg/100 ml (SIM-03) and 2448.51 mg/100 ml (SIM-06). We measured ascorbic acid between 62.87 mg/100 ml (SIM-03) and 38.15 mg/100 ml (SIM-04). Gallic acid was determined between 36.14 mg/100 ml (SIM-05) and 15.06 mg/100 ml (SIM-06).

In a research, the highest malic acid value was found as nearly 7200 mg/kg (Yayla Pınarı) and the lowest value was found as nearly 19 mg/kg (Kızıl Ahmedi) (Abacı & Sevindik, 2014). The same researchers explained that the oxalic acid was measured between 7.95 mg/kg (Kızıl Ahmedi) and 4.70 mg/kg (Uzun Yumra). The values of citric acid were measured between 55.55 mg/kg (Yayla Pınarı) and 24.10 mg/kg (Starking Delicious). The content of tartaric acid was found between nearly 380 mg/kg (Var-24) and 80.00 mg/kg (Var-YP) (Abacı and Sevindik, 2014). In apples varieties and genotypes, many researchers found that malic acid is the highest organic acid (Loue, 1968;

Lindsay & Norvell, 1978; Zhao *et al*., 1995; Kwang *et al*., 1996; Shyi & Sun 1999; Lee *et al*., 2000). Researchers found that the malic acid content of Starking apple cultivar was higher than the malic acid content of Golden cultivar. The second highest value was determined as Malonic acid and the lowest acid was Citric acid among the apple varieties (Mordoğan & Ergun, 2001). In the study conducted by Wu *et al*., (2007), the highest organic acid was found as the malic acid and the highest total organic acid value was measured at Granny Smith apple and Ralls apple. Almost the same results have been obtained in studies conducted by many different researchers and malic acid was found to have the highest value among organic acids (Hulme & Wooltorton, 1957; Ulrich, 1970; Beruter, 2004; Hecke *et al*., 2006; Petkovsek *et al*., 2007). In this study, Malic acid, Oxalic acid, Ascorbic acid, and Gallic acid were the highest organic acids, respectively. Our results are mostly in parallel with the results of other researchers and there are few differences in some values. These differences are thought to be due to genetic differences, ecological differences, and differences in analysis methods in apple cultivars.

Conclusion

Overall all, SIM-04 genotype was found to have the superior pomological characteristics and was followed by SIM-01 genotype. In terms of the pomological characteristics, the lowest scores were observed from SIM-05 and SIM-06 genotypes. Besides to that, SIM-01 genotype was also found to have superior characteristics in terms of total phenols and total antioxidant activity, but the highest total flavonoids content was noted from the SIM-06. Moreover, SIM-04 genotype was noted to have the highest values of oxalic acid, malic acid and ascorbic acid, where the highest gallic acid content was noted from the SIM-05 genotype. All of those results suggest that there is a significant difference between the pomological and chemical characteristics of the genotypes and the selection of the most appropriate genotype is highly dependent upon the preferred characteristics of the apples.

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