Morphological and Biochemical Characteristics of Wild Loquat (*Eriobotrya japonica* Lindl.) Genotypes in Turkey

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Summary

This study was carried out to determine the pomological and biochemical characteristics of eight different loquat genotypes collected from the Black Sea region (Turkey) in 2018. Totally 20 fruits, at the same ripening stage, were collected from the selected genotypes and tested. Results suggested that there were a high (0.768-0.907) positive and statistically significant correlations among all pomological features ($P < 0.01$) while the correlations among the biochemical compounds were weak and statistically non-significant ($P > 0.05$). According to the PCA (principal component analysis) analysis of the pomological characteristics, the genotype #1 was superior as compared with other genotypes. Contrary to the pomological characteristics, genotype #1 was found to have the lowest phenolic compounds and it was also moderate in sugar content but a high-grade genotype by organic acids, especially citric acid and malic acid. Furthermore, results suggested that genotypes #3, #4, #7 and #8 were identical and were rich in glucose, succinic acid, and total flavonoid. The results suggested that loquat fruits had a high potential for health benefits. The results are also a preliminary key reference for future studies in terms of loquat cultivation throughout the world and have high potential as a functional food.

Key words

hidden fruit quality, diversity, phenolic compounds, antioxidant capacity, organic acids, sugar composition

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Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a perennial plant from the Rosaceae family. The loquat trees are reported to be originated from the regions of southeastern China and Japan. These days it is also cultivated in the Mediterranean areas, Australia, South Africa, South America, California, India, and in the north of Iran (Esmaeili et al., 2013). The loquat fruit was then spread to the Mediterranean countries and Europe from its homeland. It is an important subtropical fruit tree of great value and has excellent ornamental features with a flowering period of three months, from late autumn to winter. It is also used as an excellent garden tree with green leaves (Lou et al., 2018). Subtropical fruits generally have less production area than the others and limited information is available about these species throughout the world. Especially pomegranate, persimmon, loquat, and avocado are produced and consumed in a very restricted area. In addition, loquat fruit also contains a range of bioactive phytochemicals such as phenolic compounds that can contribute to health benefits (de Faria et al., 2009). Therefore, an increase in the production and consumption of these subtropical fruits will play a significant role in people's health and agricultural prosperity. The development of subtropical fruit cultivation will also be an important source of raw materials for the food industry. In addition, revival between food and agriculture sectors will be promoted and the businesses, producing fruit juice, canned food, jam, marmalade, confectionery and ice cream, will easily reach a different kind of raw materials and will be able to expand their production capacities. Processed products of subtropical fruits are very important for increasing the marketing opportunities of fruits (Kahramanoğlu, 2019). Several studies have reported different bioactive compounds, which are likely to be associated with health-related properties of loquat. Loquat fruit is also a good source of dietary fibres and pectin and is considered beneficial for the human digestive system. It has antioxidant effects; known to prevent cell damage, protect humans against heart diseases and cancer and regulate enzyme activities. Loquat fruit is also rich in malic acid and B-group vitamins that increase the effect of antioxidants and is rich in minerals such as phosphorus, potassium and calcium and sugar. At the same time, the loquat fruit contains a high amount of vitamin A. The color of its flesh and skin is rich in carotene, a source of vitamin A in the fruit. Phytochemicals are natural chemicals that exist as protective system of the plant against diseases in vegetable products such as leaves, flowers, and roots of fruits, vegetables, medicinal and aromatic plants, pulses and cereals (Nirupama et al., 2012). This term is used to name biologically important chemical groups, including antioxidants and functional compounds consisting of phytochemicals, carotenoids, phenol, and natural indigoids, which are not essential nutrients (Beutner et al., 2001). The increasing incidence of certain chronic diseases such as cardiovascular problems, diabetes and cancer among people, has drawn attention to the importance of a healthy diet. Epidemiological studies maintained to determine the relationship between nutritional habits and disease risks prove that food type (bioactive compounds) and diet directly affect human health. This situation leads consumers to change their diet habits and provides them some additional benefits from food. Therefore, additionally to the energy and essential nutrient, phytochemicals are highly important for human physiology and metabolic functions and have positive effects on reducing the risks of many diseases. Interest in functional foods or bioactive components of these foods has been raising daily (Hardy, 2000; Roberfroid, 2000; Bekers et al., 2001; Kwak and Jukes, 2001; Stanson et al., 2005). It was observed that the essence of the loquat significantly reduced lung inflammation in alveolar macrophages and edema in the ear and rat paws, respectively. Loquat seed extracts also demonstrated in vivo anti-inflammatory effects on hamsters through chemotherapy and inhibition of allergic dermatitis-induced mucositis, epithelial lesion and bacterial infection (Huang et al., 2009; Zar et al., 2014). In line with this information, the present study aimed to determine the physical and chemical properties of loquat fruits, of which its production, trade, and consumption has recently increased throughout the world.

Material and Methods

Plant Material

Loquat fruits were collected from Trabzon province of the Eastern Black Sea region of Turkey in 2018. The altitude of this city is between 0-200 m. The latitude and longitude are 41°0'18"N, 39°43'36.98"E. Twenty fruits were selected from 8 different genotypes during the studies. Fruits were gently collected from the trees. Collected fruits were brought to the Laboratory of Uşak University (Sivaslı Vocational School) for physical measurements. After pomological measurements, juices of the fruits were squeezed with a blender, and were separated from the pulp with the help of cheesecloth. Then, loquat fruit juices were taken to the "Scientific Analysis Technological Application and Research Center (UBATAM)" for chemical analyses.

Determination of Pomological Characteristics

The average fruit weight of each genotype was calculated by using the 20 fruits. The stalks were separated from the connection point without damaging the fruit shell and flesh. Fruit weights were measured with a digital scale sensitive to ± 0.01 g. Average fruit length and width were then measured with a 0.01 mm precision digital calliper. The averages for these 20 fruits were calculated accordingly. The fruit volume (cm^3) of 20 fruits, selected from each genotype, was measured separately. The sum of the measurement carried out by overflowing the water in a 200 ml beaker is equal to the fruit volume. The average fruit volume was determined by dividing the number of fruits measured.

Determination of Total Phenolic Content

The total phenolic content of the loquat fruit extracts was determined by a spectrophotometer according to the Folin-Ciocalteu colorimetric method (Li et al., 2006). Briefly, samples of approximately 1 mL of loquat fruit extracts were taken and 60 mL of pure water was added. Then, 5 mL of Folin-Ciocalteu ready solution was added and mixed well. Next, 7.5 minutes later 15 mL of 20% sodium carbonate solution were added and the volume of the solutions was mixed to 100 mL and stirred. After centrifugation (10000 rpm, for 10 minutes at 25 °C) final solutions were stored in the dark at 25 °C for 2 hours. Hereafter, the absorbance of the solutions was read at 750 nm by a spectrophotometer. Total phenol amounts were evaluated from the calibration curve obtained with gallic acid and values to be equivalent to mg gallic acid per gram (mg GAE g^{-1}) (Li et al., 2006).

Determination of Total Flavonoids Content

For the analysis of total flavonoid substance (TF) amount the spectrophotometric method of Christ and Müller (Polish Pharmacopoeia, 2006) was used after their extraction, as recommended by the European Pharmacopoeia (European Pharmacopoeia, 2013). For this purpose, 5.0 g of crushed fruit were added to a round-bottomed flask; 20 mL of acetone, 2 mL of HCl (281 g L^{-1}), and 1 mL of methenamine (5 g L^{-1}) were then added and the mixture was maintained for 30 min under reflux on a water bath. The hydrolysate was filtered through cotton wool into a volumetric flask of 100 mL, then placed in a flask together with the cotton pellet and 20 mL of acetone, and refluxed for 10 min. Next, 20 mL of solution were dispensed into a separatory funnel with 20 mL of water and extracted with ethyl acetate in 15 mL portions 3 times with 10 mL. The combined organic layers were washed twice with 40 mL of water, filtered into a volumetric flask of 50 mL, and supplemented with ethyl acetate. To determine flavonoid content, two samples were prepared: to 10 mL of the stock solution 2 mL of a solution of aluminum chloride (20 g L^{-1}) were added, supplemented with a mixture (1 : 19) of acetic acid (1.02 kg L^{-1}) and methanol (25 mL). To prepare the comparative solution, stock was supplemented with 10 mL of a mixture (1 : 19) of acetic acid (1.02 kg L^{-1}) and methanol (25 mL). After 45 min, the absorbance of the solutions was read at λ = 425 nm on HITACHI U-2900 spectrophotometer using the reference solution for comparison. Samples were analyzed in 3 replicates. The content TF (mg g^{-1}) was expressed as quercetin equivalent (QE) according to the following formula:

$$X = (k \cdot A)/m$$

where total flavonoids (mg g^{-1}) are; the absorbance of the solution is being studied; the convection factor for quercetin and equal to 8.750; is the sample with the raw material (g) which was the amount of fresh material.

Determination of Antioxidant Capacity

The free radical removal properties of loquat fruit extracts were determined by using 2,2-diphenyl-1-picrilhydrazil (DPPH), according to the method of Singh et al., (2002) with minor modifications. For this purpose, 0.1 mL of each extract was taken, and then a solution of DPPH prepared in 4.9 mL ethanol (0.1 M) was added. After 30 minutes at room temperature and keeping in the dark for 2 hours, the UV / VIS spectrophotometer (Shimadzu UV-1800, Japan) was used to read the absorbance at 517 nm. The control was prepared in the same reaction mixture using methanol (80%) instead of plant extract with the same procedure (Ismail et al., 2010). The percentage of reduction of the sample as compared to standard (methanol) was calculated using the following formula:

$$\text{DPPH - of reduction power} = ((Ac\text{-}As/Ac)) \times 100$$

In the formula given above, Ac is the absorbance of control, As represents the absorbance of sample and the Ab equals to the absorbance of blank.

Sugar Analysis

Sugar (fructose, sucrose, and glucose) analysis was performed with the methods described by Melgarejo et al. (2000) and Gecer, et al., (2016), respectively, with minor modifications. 1 ml of fruit extracts was centrifuged at 10 000 rpm for 2 min at 4 °C. Supernatants were passed by SEP-PAK C18 cartridge. HPLC readings were made with µbondapak- NH_2 column using 85% acetonitrile as the liquid phase with a refractive index detector (IR). Fructose and glucose standards were used for the calculations of the sugar contents.

Organic Acids Analysis

Malic acid, citric and succinic acid composition of the loquat fruit were determined with the methods described by Bevilacqua and Califano (1989) and Gecer et al. (2016) with minor modifications. Fruit extracts were obtained by crushing the fruits in cheesecloth. 0.009 NH_2SO_4 was then homogenized with shaker for 1 h. The mixture was then centrifuged at 15000 rpm for 15 min and the supernatants were filtered twice through a 0.45 µm membrane filter with a coarse filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) and passed through a SEP-PAK C18 cartridge. Organic acid readings were performed by HPLC using the Aminex column (HPX-87 H, 300 mm x 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) at 214 and 280 nm wavelengths in the Agilent package program (Agilent, USA).

Analysis of Ascorbic Acid

Ascorbic acid content was determined by the modified HPLC-DAD procedure proposed by Święciło et al. (2018). 5 mL of fruit extracts were supplemented with 2.5% (w / v) metaphosphoric acid (Sigma, M6285, 33.5%), then centrifuged at 5500 rpm for 15 minutes at 4 °C. Then, a 0.5 mL sample of the mixture were raised to 2.5 mL (w / v) metaphosphoric acid to a final volume of 10 mL. The supernatants were filtered through a 0.45 PTm PTFE syringe filter (Phenomenex, UK). A C18 column (Phenomenex Luna C18, 250 mm x 4.60 mm, 5 umm) was used at 25 °C to identify ascorbic acid. It was used as a mobile phase at a flow rate of 1 mL min^{-1} and pH 2.2 (ultra distilled with distilled H_2SO_4). Spectral measurements were made at 254 nm using a DAD detector. Different L-ascorbic acid standards (SigmaA5960) (50, 100, 500, 1000 and 2000 ppm) were used for the measurement of ascorbic acid readings.

Statistical Analyses

Non-parametric tests were used for the statistical comparison of the study parameters while the observations did not provide the normal distribution assumption for each genotype. Descriptive statistics are given as mean, standard deviation, median and the interquartile range since the data are continuous variables. Differences between genotypes were examined by Kruskal Wallis test. Correlations between variables were analysed using Pearson correlations. In order to reveal the similarities and differences between the genotypes and variables, the principal component analysis was applied. The level of statistical significance was determined at 0.05 and 0.01 levels. Statistical analyses were performed using SPSS 24 and R 3.5.1 software.

Results and Discussion

Identification and Quantification of Pomological Characteristics and Bioactive Components

The relationship among the pomological characteristics and bioactive contents of the tested loquat genotypes are given in Table 1. Results suggested that there are high (0.768-0.907) positive and statistically significant correlations among all pomological features ($P < 0.01$). On the other hand, correlations among the chemical compounds were found to be weak and statistically non-significant ($P > 0.05$). However, there was a significant correlation between total phenol and fruit width (-0.545), while the flavonoid content was found to be negatively and highly correlated with all pomological features ($P < 0.01$). The correlations between sugar components were not statistically significant ($P > 0.05$). A significant positive correlation was found among fruit weight, glucose and fructose contents ($P < 0.05$). The fruit length was negatively correlated with glucose content ($P < 0.05$). Moreover, glucose content was positively correlated with total flavonoid (0.637) and fructose content was negatively correlated with phenol and DPPH (-0.613 and 0.637) ($P < 0.01$). There was a positive and significant correlation between glucose content with malic acid and with succinic acid (0.536 and 0.647) ($P < 0.05$). Ascorbic acid was found to be negatively correlated with citric, malic and succinic acid, but it had a positive correlation with malic acid and citric acid.

Pomological Characteristics

Results of the pomological characteristics of the 8 loquat genotypes are given briefly in Table 2. A statistically significant difference was found between genotypes in terms of fruit weight at $P < 0.05$ level. The results of the present study showed that there was a significant difference among all genotypes in fruit weight. The highest fruit weight was related to genotype #1 and the lowest fruit weight was recorded at genotype #4 ($P < 0.05$). Fruit length was also found to be significantly varied among the genotypes ($P < 0.01$). The shortest fruits were noted from the genotype #7 and the highest fruit length was noted from genotype #1. Not surprisingly, genotype #1 was also noted to have the highest fruit width and volume as well as fruit weight and fruit length. However, genotypes #2, #5 and #8 were statistically similar to genotype #1 ($P > 0.05$) in terms of fruit length. A statistically significant difference was found between genotypes in terms of fruit width ($P < 0.01$). Similarly to the fruit length and weight, the most average of fruit width was observed in genotype #1 and the lowest average of fruit width was obtained from genotypes #3 and #4. However, genotypes #5 and #6 were statistically similar to genotype #1 ($P > 0.05$). Similar to other characteristics, fruit volume was also found to have a statistically significant difference among different genotypes ($P < 0.01$). Genotypes #1, #2 and #5 were found to have the highest average of fruit volume. The lowest fruit volume was then observed in genotype #4 and was statistically different from other genotypes ($P < 0.05$).

Table 1. Pearson Correlation Coefficients in determining relationships between chemical compounds in Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

	Weight	Length	Width	Fruit volume	TP	TF	DPPH	Glucose	Fructose	Sucrose	Citric Acid	Malic Acid	Ascorbic Acid
Length	0.785**												
Width	0.805**	0.871**											
Fruit volume	0.907**	0.768**	0.780**										
TP	-0.358	-0.183	-0.545**	-0.119									
TF	-0.796**	-0.904**	-0.827**	-0.818**	0.196								
DPPH	-0.279	0.026	-0.101	-0.09	0.362	-0.252							
Glucose	-0.615**	-0.521**	-0.392	-0.422*	0.212	0.637**	-0.165						
Fructose	0.559**	0.025	0.35	0.435*	-0.613**	-0.083	-0.695**	-0.110					
Sucrose	-0.111	-0.028	-0.303	0.139	0.779**	-0.045	0.248	0.018	-0.307				
Citric Acid	0.189	0.585**	0.559**	0.249	-0.201	-0.436*	-0.068	0.134	-0.121	0.071			
Malic Acid	-0.295	-0.029	0.178	-0.163	-0.273	-0.024	0.176	0.536**	-0.109	-0.148	0.632**		
Ascorbic Acid	0.354	0.187	-0.024	0.430*	0.471*	-0.391	0.408*	-0.492*	-0.096	0.321	-0.522**	-0.564**	
Succinic Acid	-0.446*	-0.181	-0.34	-0.338	0.460*	0.467*	-0.333	0.647**	-0.304	0.404	0.395	0.207	-0.479*

*$P < 0.05$, **$P < 0.0$

Table 2. Physicochemical characteristics of fruit Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

	Variables							
	Weight (g)		Length (mm)		Width (mm)		Fruit Volume (cm^3)	
Genotypes	Mean ± S.D.	Median (IQR)	Mean±S.D.	Median (IQR)	Mean±S.D.	Median (IQR)	Mean ± S.D.	Median (IQR)
1	40.31±0.32	40.43(0.61)^{A}	50.92±0.31	50.87(0.61)^{A}	44.77±0.35	44.73(0.69)^{A}	47.50±2.09	46.78(3.99)^{A}
2	33.16±1.41	32.49(2.57)^{B}	42.66±0.39	42.77(0.76)^{AB}	36.54±0.34	36.53(0.67)^{CD}	34.72±0.55	34.96(1.01)^{A}
3	29.29±0.41	29.14(0.78)^{BC}	32.75±0.41	32.79(0.81)^{C}	35.64±0.30	35.49(0.54)^{D}	29.33±1.03	29.86(1.84)^{BC}
4	22.55±0.89	22.43(1.77)^{D}	33.80±0.38	33.81(0.76)^{C}	35.35±0.51	35.14(0.96)^{D}	25.15±0.85	25.11(1.69)^{C}
5	34.17±1.19	34.04(2.38)^{B}	43.06±1.01	42.78(1.96)^{AB}	38.65±0.44	38.84(0.82)^{AB}	46.57±0.33	46.61(0.66)^{A}
6	27.98±0.34	28.14(0.62)^{C}	42.58±0.37	42.66 (0.73)^{AB}	38.73±0.66	38.44(1.23)^{AB}	29.77±0.41	29.59(0.75)^{BC}
7	26.07±0.78	25.77(1.47)^{C}	37.44±0.3	37.36(0.57)^{B}	35.85±0.58	35.88(1.17)^{CD}	29.57±0.37	29.54(0.74)^{BC}
8	27.27±0.75	27.38(1.48)^{C}	42.81±0.28	42.87(0.54)^{AB}	37.26±0.75	36.88(1.36)^{BC}	30.64±0.40	30.84(0.71)^{B}
Avarage	**30.10±5.36**	**28.59 (6.83)**	**40.75±5.65**	**42.37 (7.26)**	**37.85±2.98**	**36.83 (2.74)**	**34.16±8.03**	**30.21 (10.97)**
Kruskal Wallis H	22.267		20.239		21.239		21.187	
P-value	0.02		0.005		0.003		0.004	

A, B, C, D ...– values in columns denoted by the same small letters are not statistically different at $P < 0.05$

In previous research, Senyurt (2006) conducted a study with the local loquat genotypes grown in Ordu and found that the average of fruit weight was between 26.66 - 60.29 g. In other studies, fruit weights were recorded between 20.45 g and 36.12 g (Paydaş et al., 1992). In another similar research, Hermoso and Farré (2003) performed a study in Spain and reported that the fruit weight of the Gold Nuget loquat variety varied from 39 to 45 g. Researchers previously have found that the fruit length of loquat varies between 29.86 - 45.89 mm (Şenyurt, 2006; Polat, 2007; Topuz, 1998). Many researchers previously reported that the fruit width also varied among genotypes and could be between 29.80 - 53.81 mm (Llácer et al., 2003; Durgac et al., 2006; Polat, 2007). Results of these previous studies are all in agreement with the results of the present study, in which the fruit weight, fruit length, fruit width, and fruit volume significantly vary among the different genotypes. Moreover, the results are in conjunction with the unit values reported by previous researchers.

The PCA (principal component analysis) graph of pomological characteristics and genotypes of the present study is given in Fig. 1. The cross-evaluation of Fig. 1. and Table 1., shows that genotype #1 is superior in terms of pomological characteristics compared to other genotypes. The genotypes closest to this genotype are found to be genotypes #2 and #5, with the worst pomological genotype being genotype #4. High correlations between the variables are also seen on the PCA graph.

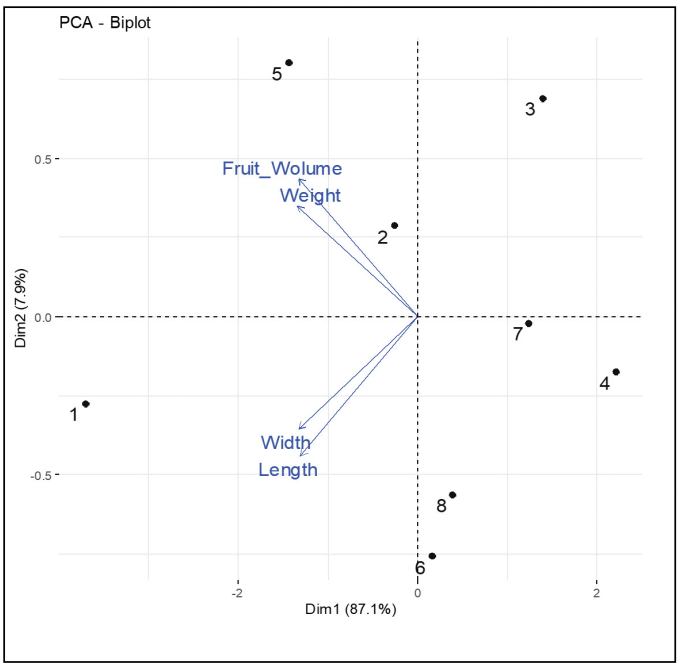

Figure 1. PCA graph of pomological characteristics of Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

Bioactive Components

Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity

Descriptive statistics and comparison of total phenolic (TP), total flavonoid (TF) and total antioxidant capacity (TAC (DPPH)) of genotypes are given in Table 3. A statistically significant difference was found among genotypes in terms of all three parameters ($P < 0.01$). Results showed that the TP, TF and TAC contents ranged from 3123.94 to 5008.23 mg GAE L^{-1}; 1247.57 to 2228.42 mg GAE L^{-1} and 2690.47 to 3488.91 mg GAE L^{-1}, respectively. Results also showed that contrary to the pomological characteristics, the lowest TP was obtained from genotype #1 (which is superior in terms of pomological characteristics) and the highest TP was noted from genotype #5. The fact that the genotype with high fruit weight had the lowest TP can be explained by the high negative correlation between these two variables. Moreover, in terms of TF, genotype #7 was the highest genotype and genotypes #1 and #5 were the lowest. The highest genotypes in terms of TAC were #6, and the lowest, #1, #3 and #7 (Table 3. and Fig. 2.). Numerous studies reported that the fruits antioxidant activity is probably due to the effect of phenolic compounds. Many previous studies have already observed a wide change in the TP content at different loquat varieties. The results of those studies ranged from 818 mg g^{-1} and 1738 mg g^{-1} (Ding et al., 2001), 125.7 to 2603.3 µg GAE g^{-1}, (Ferreres et al., 2009), 129 to 578 µg GAE g^{-1} (Polat et al., 2010), 240 to 572 µg GAE g^{-1} (Xu and Chen 2011), 140 to 753 µg GAE g^{-1} (Ercisli et al., 2012) 394.67 to 664.53 µg GAE g^{-1} (Delfanian et al., 2015) and 2954.50 – 5071.62 mg gallic acid kg^{-1} (Erkolencik, 2016). Similar with the TP contents, the TF contents of the loquat fruits were previously reported to vary among different varieties. In some of these studies, it was noted that the TF ranged from 21.22 to 77.5 mg g^{-1} (Xu and Chen 2011), 16.3 to 38.7 µg g^{-1} (Ercisli et al., 2012) and 1189.01 to 2020.78 mg L^{-1} (Erkolencik, 2016). Antioxidant capacities can be affected by several factors and cannot be fully defined by a single method. Therefore, antioxidant capacities were measured using DPPH in this study. Hong-Xia et al. (2014) previously reported that the values of total phenolic varied from 0.66 to 0.96 mg g^{-1} GAE, total flavonoid amount ranges from 0.09 to 0.21 mg g^{-1}, and the total antioxidant capacity (DPPH) varied from 2.91 to 4.93 µmol TE g^{-1} in six loquat cultivars. Zhang et al. (2015) also previously conducted a study with the DPPH method for the determination of antioxidant substance analysis at the fruit shell of seven loquat cultivars. They reported that the highest DPPH activity was noted from the "Dahongpao" cv with 36.64 mg TED g^{-1} and the lowest value was noted from "Jiajiao" cultivar with 25.19 mg TED g^{-1}. Results of present study for the total phenolic content, total flavonoid and total antioxidant activity were inconsistent with the previously reported results of Koba et al., (2007), Ferreres et al., (2009), Ercisli et al., (2012), Xu et al., (2014) and Erkolencik, (2016). It was also previously reported that TP content, TF content and TAC of the loquat fruits might vary according to the growth, maturation stage, genetic factors of each cultivar, environmental conditions and extraction methods (Zhou et al., 2011).

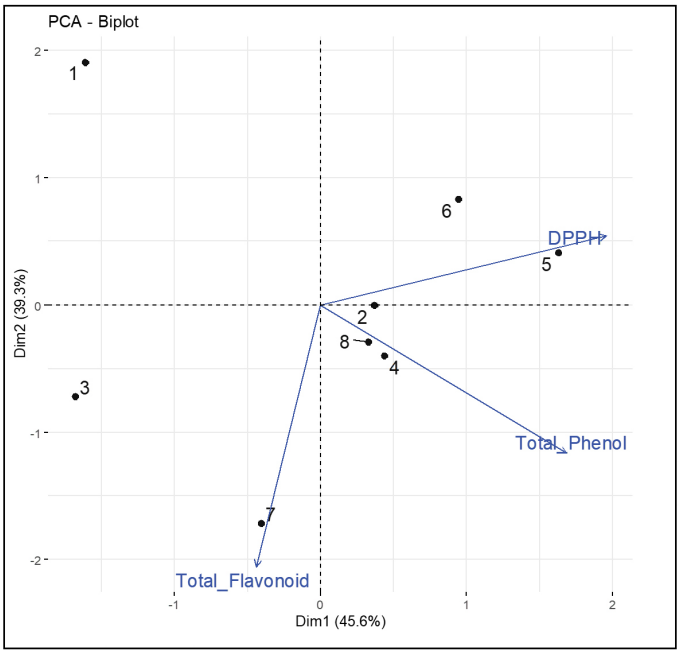

Figure 2. PCA graph of chemical characteristics of of Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

The PCA graph of chemical properties showed that the most prominent genotype (genotype #1) for pomological characteristics was found to have the lowest phenolic compounds and was followed by genotype #3 (Table 3.). Genotypes #5 and #6 are found to be genetically rich in DPPH, genotype #7 is rich in total phenol and genotypes #2, #4 and #8 are found to be rich in flavonoids. There was no strong relationship between these three variables.

Sugar Contents

Sugar is an energy source of the plants and harvested fruits and is broken down by biosynthesis pathways (mainly respiration) to produce energy when cells need it. Sugars such as sucrose, glucose and fructose are not only the most important factors affecting fruit taste and quality (Itai and Tanahashi 2008). The sugar concentrations (mg g^{-1}) of different loquat genotypes are given in Table 4. and Fig. 3. The illustrations indicate that the amount of sucrose, glucose, and fructose concentrations are significantly different among different genotypes ($P < 0.01$). Results showed that the sucrose was the high and predominant sugar for all genotypes. Sucrose content was slightly higher than glucose and fructose for all genotypes (Table 4). Genotype #4 had the highest sucrose content (3.48 mg g^{-1}) while genotype #1 had the lowest (1.79 mg g^{-1}). The highest glucose content was found in genotypes #4 and #7 (1.46 and 1.51 mg g^{-1}), and the lowest values were recorded from genotypes #2 and #6 (0.78 and 0.87 mg g^{-1}). Genotypes #1 and #3 had the highest fructose content and genotypes #6 and #8 had the lowest fructose contents. In terms of sucrose, genotypes 5 and 8 were found to have the highest content and genotypes #1 and #6 were with the lowest values (Table 4. and Fig. 3.). Similarly, it was previously reported that sucrose was the dominant sugar in loquat fruits (Topuz, 1998; Bantog et al., 1999; Hasegawa et al., 2010). On the contrary, some of the previous studies reported opposite results, i.e.: Xu and Chen (2011) reported that fructose was predominant in loquat fruits. They also reported that glucose and fructose contents of 12 different loquat varieties were higher than the result of current work which quantified from 30.0 mg g^{-1} and 53.6 mg g^{-1}, and 35.9 mg g^{-1} and 54.2 mg g^{-1}respectively.

Table 3. Content of selected secondary metabolites and antioxidant activity of fruit extracts of Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

	Variables					
	TP (mg GAE/g)		TF (QE/mg)		TAC (% DPPH)	
Genotypes	Mean±S.D.	Median (IQR)	Mean±S.D.	Median (IQR)	Mean±S.D.	Median (IQR)
1	3123.94±1.66	3124.15(3.31)^{F}	1247.57±2.58	1248.63(4.83)^{E}	2723.37±1.97	2723.91(3.83)^{D}
2	4656.34±3.42	4657.96(6.25)^{B}	1637.13±1.96	1638.19(3.47)^{D}	2983.2±2.5	2982.45(4.84)^{C}
3	3587.51±0.42	3587.44(0.83)^{E}	2157.38±3.51	2159.31(6.16)^{B}	2690.47±1.65	2690.16(3.26)^{D}
4	4236.00±1.27	4236.59(2.32)^{C}	2003.49±2.06	2003.63(4.12)^{C}	3252.99±3.18	3251.15(5.51)^{B}
5	5008.23±9.61	5004.56(18.14)^{A}	1464.4±3.44	1462.62(6.14)^{E}	3314.89±2.33	3315.78(4.40)^{AB}
6	3982.90±0.63	3982.66(1.19)^{D}	1653.94±4.11	1652.92(8.02)^{D}	3488.91±1.33	3489.00(2.66)^{A}
7	4752.30±0.28	4752.18(0.51)^{B}	2228.42±11.08	2234.78(19.22)^{A}	2752.04±1.57	2751.15(2.73)^{D}
8	4632.97±5.03	4632.56(10.04)^{B}	1765.42±3.45	1763.54(6.07)^{D}	3001.99±0.85	3001.81(1.67)^{C}
Total	**4247.52±616.88**	**4432.51(920.2)**	**1769.72±325.51**	**1710.9(527.8)**	**3025.98±286.87**	**2993.62(546.39)**
Kruskal Wallis H	22.680		22.680		22.690	
P-value	0.002		0.002		0.002	

A, B, C, D ...– values in columns denoted by the same small letters are not statistically different at $P < 0.05$

Table 4. Qualitative and quantitative composition of identified sugars (mg / g) in the fruits of Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

	Variables						
	Glucose		Fructose		Sucrose		Total sugar
Genotypes	Mean ± S.D.	Median (IQR)	Mean±S.D.	Median (IQR)	Mean ± S.D.	Median (IQR)	
1	0.99±0.03	0.98(0.06)^{C}	1.8±0.05	1.82(0.09)^{A}	1.79±0.01	1.79(0.02)^{C}	4,58
2	0.78±0.01	0.78(0.01)^{E}	1.27±0.03	1.28(0.05)^{BC}	2.58±0.04	2.58(0.07)^{AB}	4,63
3	1.03±0.02	1.04(0.04)^{BC}	1.86±0.06	1.87(0.12)^{A}	2.27±0.07	2.25(0.14)^{BC}	5,16
4	1.46±0.04	1.48(0.07)^{A}	1.21±0.04	1.21(0.07)^{C}	2.35±0.07	2.35(0.14)^{BC}	5,02
5	1.02±0.03	1.02(0.06)^{BC}	1.38±0.02	1.38(0.04)^{AB}	3.48±0.12	3.50(0.23)^{A}	5,88
6	0.87±0.02	0.87(0.03)^{D}	0.96±0.01	0.96(0.01)^{D}	1.99±0.04	2.00(0.07)^{C}	3,82
7	1.51±0.05	1.52(0.10)^{A}	1.25±0.04	1.25(0.07)^{BC}	2.38±0.05	2.39(0.09)^{BC}	5,14
8	1.12±0.02	1.12(0.03)^{AB}	1.07±0.02	1.07(0.04)^{D}	3.37±0.09	3.40(0.16)^{A}	5,56
Avarage	**1.10±0.25**	**1.03(0.36)**	**1.35±0.31**	**1.27(0.44)**	**2.53±0.58**	**2.37(0.83)**	
Kruskal Wallis H	21.885		21.885		21.885		
P-value	0.003		0.003		0.003		

A, B, C, D ...– values in columns denoted by the same small letters are not statistically different at $P < 0.05$

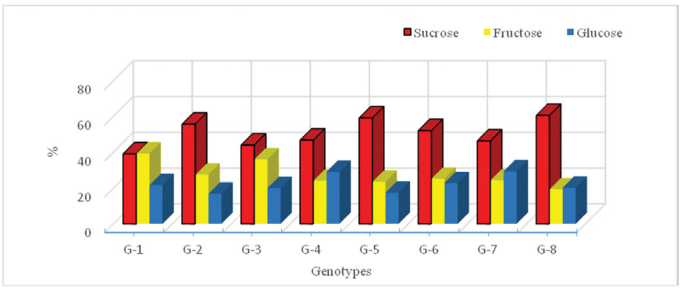

Figure 3. Organic sugars detected in Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes fruit concentrates as the percentage of the total organic sugars

Hasegawa et al. (2010) previously reported similar findings with this present study for different loquat cultivars where they had found glucose between 0.50 to 1.51 mg g^{-1}, fructose from 0.89 to 1.82 mg g^{-1} and sucrose from 2.70 to 4.96 mg g^{-1}.

The PCA graph of sugar compounds and genotypes is given in Fig. 4. Distribution of genotypes in terms of sugar content show that the genotypes #2, #6, #5 and #8 are identical for sucrose content, where genotypes #1 and #3 are identical for fructose and finally the genotypes #4 and #7 are identical for glucose contents. Although it is not statistically significant in correlation analysis, it is clearly observed from the PCA graph that fructose is negatively associated with glucose and sucrose contents.

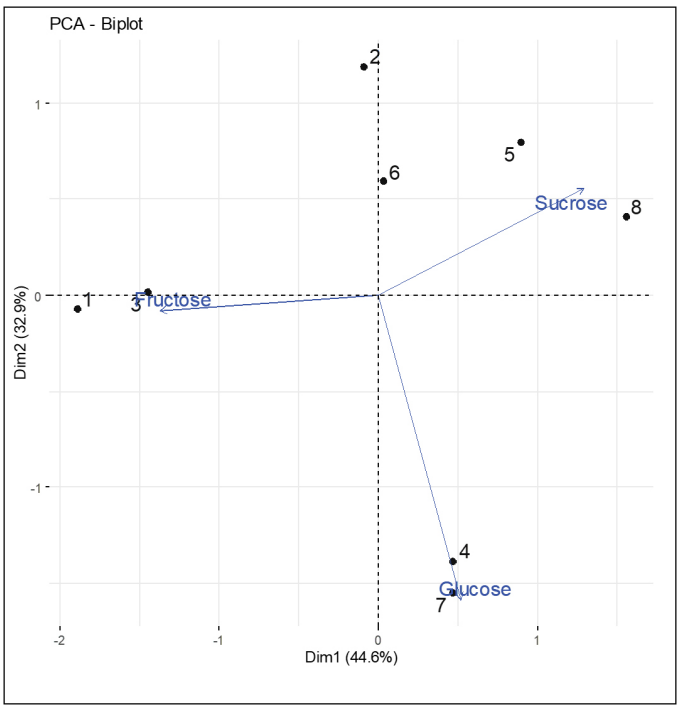

Figure 4. PCA graph of sugars characteristics of in Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

Organic Acids and Ascorbic Acid

Organic acids are known to particularly affect the fruits' taste formation and many physiological processes. A statistically significant difference was found between genotypes in terms of organic acids and ascorbic acid ($P < 0.01$) (Table 5. and Fig. 6.). Fig. 5 depicts individual organic acids expressed as a percentage of the total organic acids. Results showed that organic acid compounds of the genotypes varied significantly and the most predominant organic acids in these loquat genotypes was malic acid generally followed by citric and succinic acids. Some similar studies previously reported that the malic acid was the predominant organic acid in loquat fruits (Chen et al., 2008; Hasegawa et al., 2010; Toker et al., 2013). The malic acid levels ranged from 360.73 to 645.01 mg g^{-1} among the test genotypes of the present study. Malic acid was found to be the highest in genotype #4 where genotypes #1 and #8 were similar to genotype #4 in terms of malic acid content. The lowest malic acid was found in genotype #2 (Table 5. and Fig. 5.).

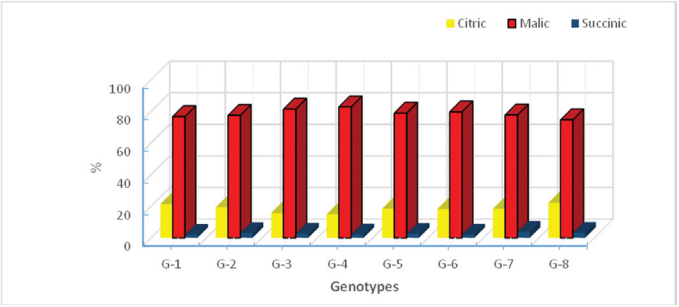

Figure 5. Organic acids detected in in Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes fruit concentrates as the percentage of the total organic acids

The highest citric acid concentration was then measured from genotypes #1 and #8 and the lowest values were recorded from genotypes #2 and #3. Genotypes #2 and #5 were then found to have the highest ascorbic acid concentrations. Other genotypes were found to have significantly lower ascorbic acid concentrations than genotypes #2 and #5, but no significant differences were found among them. Genotypes with the highest value in terms of succinic acid were genotypes #7 and #8. These two genotypes were statistically different from other genotypes in terms of succinic acid and the remaining six genotypes contained the same level of succinic acid. Different researchers have previously reported that malic acid concentrations varies among different cultivars from 250 to 850 mg g^{-1} (Serrano et al., 2003), from 129.9 to 891.2 mg g^{-1} (Chen et al., 2008), from 587.97 to 988.05 mg g^{-1} (Hasegawa et al., 2010) and from 68.56 to 842.49 mg g^{-1} (Toker et al., 2013). The ascorbic acid content of loquat fruit genotypes was high and ranged from 22.64 to 42.75 mg g^{-1} (Table 5). Hasegawa et al. (2010) previously reported that ascorbic acid concentration varied from 5.28 to 8.20 mg g^{-1}. In another study, Ercisli et al., (2012) reported that ascorbic acid concentration of different loquat varieties varied from 4.17 to 8.33 mg g^{-1}. The results of the present study are inconsistent with the results of these previous studies. In addition to the genotype, the differences in the concentrations of many organic acids are related to ecology, cultivation techniques and date of harvest.

The relationship between genotypes and organic acids is presented in the PCA graph (Fig. 6.). Results suggest that the genotypes #1 and #4 are identical for citric acid and malic acid concentration where genotypes #2, #3, #5 and #6 are similar in terms of ascorbic acid, and genotypes #7 and #8 are identical for succinic acid content.

Table 5. Qualitative and quantitative composition of organic acids (mg/g) in the fruit of Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

	Variables							
	Citric Acid		Malic Acid		Ascorbic Acid		Succinic Acid	
Genotypes	Mean ± S.D.	Median (IQR)	Mean±S.D.	Median (IQR)	Mean±S.D.	Median (IQR)	Mean ± S.D.	Median (IQR)
1	151.46±3.92	153.19(7.25)^{A}	546.68±19.12	544.67(38.08)^{B}	26.4±0.92	26.87(1.66)^{B}	14.08±0.49	14.33(0.88)^{B}
2	89.70±1.58	90.05(3.11)^{C}	360.73±14.27	358.88(28.37)^{D}	41.91±0.3	41.95(0.59)^{A}	14.71±0.19	14.80(0.34)^{B}
3	80.39±0.60	80.73(1.04)^{C}	417.06±12.47	412.32(23.55)^{C}	26.61±0.33	26.59(0.66)^{B}	14.19±0.18	14.18(0.35)^{B}
4	116.27±1.58	115.93(3.11)^{B}	645.01±5.15	646.88(9.77)^{A}	27.57±0.36	27.76(0.64)^{B}	16.67±0.26	16.78(0.49)^{B}
5	102.12±3.16	101.43(6.21)^{B}	436.37±11.93	438.08(23.68)^{C}	42.75±1.88	42.10(3.59)^{A}	14.29±0.20	14.20(0.36)B
6	106.95±2.15	107.64(4.14)^{B}	468.61±6.54	466.24(12.42)^{C}	31.25±0.49	31.46(0.91)^{AB}	12.07±0.41	12.05(0.81)^{B}
7	105.57±3.11	105.57(6.21)^{B}	447.69±5.41	450.24(9.88)^{C}	26.8±0.37	26.63(0.68)^{B}	22.35±0.16	22.37(0.32)^{A}
8	156.64±5.21	157.33(10.35)^{A}	530.58±22.54	519.84(41.05)^{B}	22.64±0.76	22.60(1.52)^{B}	22.80±1.00	22.45(1.92)^{A}
Total	**113.64±26.18**	**106.61(37.26)**	**481.59±85.81**	**457.47(109.21)**	**30.74±7.22**	**27.19(9.87)**	**16.4±3.85**	**14.5(5.27)**
Kruskal Wallis H	21.885		22.267		21.187		21.187	
P-value	0.003		0.002		0.004		0.004	

A, B, C, D ...– values in columns denoted by the same small letters are not statistically different at $P < 0.05$

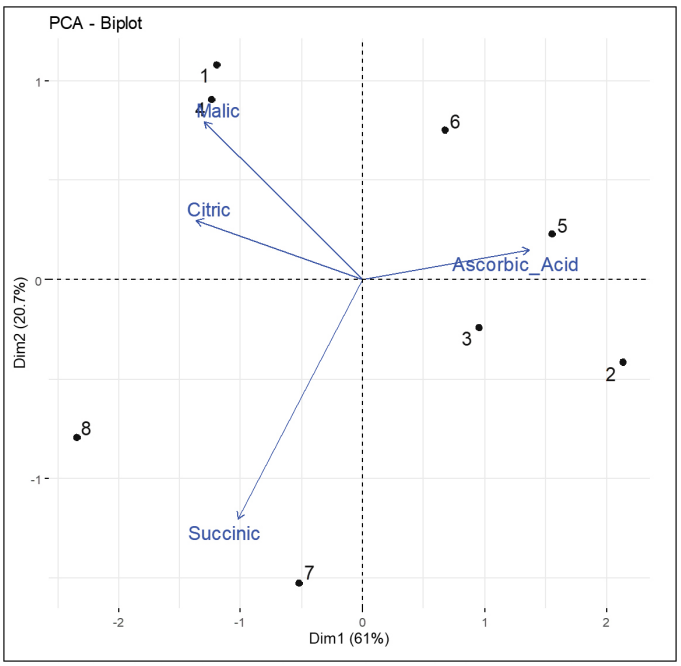

Figure 6. PCA (principal component analysis) graph of organic acids characteristics in Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

It is evident that ascorbic acid is negatively correlated with other acids and there is a high positive correlation between malic acid and citric acid.

All pomological characteristics, bioactive compounds, sugars contents, organic acids and ascorbic acid concentrations were handled together with genotypes and presented in the PCA graph (Fig. 7.). It is clear from the PCA graph that genotypes #3, #4, #7 and #8 are identical and rich in glucose, succinic acid, and total flavonoid. However, these genotypes are at the same time found to have the lowest values in terms of pomological characteristics. Genotypes #2, #5 and #6 constitute another similar genotype group. These genotypes are rich in total phenol, DPPH, sucrose, and ascorbic acid. Pomological characteristics of these genotypes are moderate where malic acid, citric acid, succinic acid, glucose, fructose, and flavonoid contents are low. Genotype #1 is a genotype that differs from other genotypes and has the best pomological properties and lowest chemical properties. However, it is moderate in sugar content and is a high-grade genotype by organic acids, especially citric acid, and malic acid.

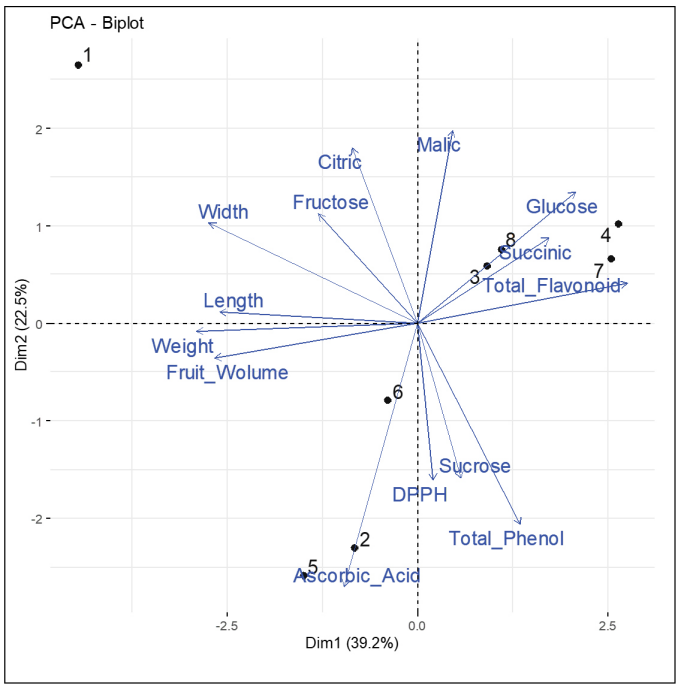

Figure 7. PCA (principal component analysis) graph of all variables characteristics in Wild Loquat (*Eriobotrya japonica* Lindl.) genotypes

Conclusions

Nowadays, not only the fruit appearance, taste and market price but also the health concern due to the vital nutrient content of the fruits is of great importance on consumer preferences. There are limited numbers of studies in the literature on the morphological and biochemical contents of the loquat fruit. In particular, this study included a number of loquat species and is therefore considered to be a valuable reference for future studies on the morphological and biochemical properties of genotypes to find the most suitable for commercial production. The results show that all of the tested loquat genotypes can be demonstrated in the natural consumption of fruits, particularly genotypes #1, #2, #4 and #5 which presented better characteristics especially for the chemical properties and pomological characteristics. The results also suggest that loquat fruits have a high potential for health benefits according to lots of studies implying on their anti-inflammatory and medicinal effect.

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