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Proximate biochemical parameters and antioxidant capacity of eight loquat genotypes (*Eriobotrya Japonica Lindl.*) from Zegzel Valley of Morocco

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The loquat fruit is a very precious product due to its economic and health benefits. For this reason, the evaluation of their biochemical profile and antioxidant activity has become required. Indeed, healthy fruits from eight loquat genotypes belonging to four regions of the Zegzel Valley are analysed. As a result, Moroccan loquat fruit showed a great pomological and biochemical richness. From the eight genotypes, the 'Z1' produced the biggest fruits (54.55 g) while 'TA6' had the highest sugar content (54.85 mg. g^{-1}). The genotypes of 'TA5', 'TA6', and 'TA7' showed the highest levels of fructose (31.64 mg. g^{-1}), sucrose (21.09 mg. g^{-1}), and glucose (10.34 mg. g^{-1}) respectively. In addition, the 'T2' showed the highest content of flavonoids (59.58 µg RE. g^{-1}), vitamin C (14.19 mg AAE/100g), and organic acids which are malic acid (193.75 mg/100 g), quinic acid (107.25) and succinic acid (12.6 mg/100 g). The total phenolics are abundant in 'TZN1' (186.0.5 µg GAEg-1), while the greatest carotenoid content was revealed in 'TA7' (90.65µg ß-carotene g^{-1}). The proteins and tannins content, seem to be similar in all genotypes (0.98 g/100g, 2.94-2.61 µg. g^{-1} respectively). Regarding the DPPH, ABTS, and FRAP tests, the 'Z7' recorded the highest antioxidant capacity compared to the other genotypes. Overall, the quantity of major bioactive compounds and antioxidant capacity varied considerably among the eight genotypes. According to the results obtained in this study, the eight loquat genotypes have a great pomological and biochemical potential that can be exploited in vegetative propagation and improvement programs of this tree.

1. Introduction

The loquat (*Eriobotrya japonica* (Thunb.) Lindl.) is a subtropical evergreen tree of the *Rosaceae* family (Sun et al., 2020). The origin of loquat has been approved as the middle and lower valley of the Daduhe River in China (Zhang et al., 1993), but it was subsequently cultivated commercially in many other countries, including Japan, Brazil and northern India (Chen et al., 2009). In the Mediterranean region, Spain and Turkey are the countries where the cultivation of this species has developed successfully over the last 20 years due to the most suitable ecological conditions for the growth of loquat and its early production. (Virginia et

al., 2011). The loquat fruit is widely consumed as fresh fruit or juice for its excellent flavour and abundant nutritional values, which may protect against inflammation, diabetes, cancer, bacterial infections, aging, pain, allergies, and other health problems (Kim and Shin, 2009; Sun et al., 2020; Liu et al., 2016). The loquat has been introduced in Morocco from Algeria by French colonization at the beginning of the century (Rhomari, 2013). The valley of Zegzel, located in the northwestern region of Morocco, accounts for 85% of the country's loquat-growing area. This area produces mainly four varieties, which are Tanaka (Japanese cultivar) and three others known locally as Navela, Muscat, and Mkerkebe. In 2021, the national production of loquat has exceeded 10,000 tons, with a significant improvement in the size and gustative quality of this exceptional local product (RDAO, 2021). Most of the studies carried out on the loquat fruit-focused mainly on its phenotypic characterization, while research on bioactive compounds and antioxidant capacity is restricted. The analysis of the organic acid and sugar composition of loquat cultivars indicated that malic acid is the predominant organic acid, followed by tartaric, quinic, citric, succinic, fumaric, and oxalic acids. In addition, fructose and glucose are the most abundant sugars, and the others are sucrose, maltose, and sorbitol (Serrano et al., 2003; Tian et al., 2007; Amoros et al., 2008; Chen et al., 2009; Pande & Akoh, 2010; Xu et al., 2010). Furthermore, the investigations have demonstrated the high antioxidant capacity of loquat extracts in *vitro* and in *vivo* using multiple antioxidant tests (Liu et al., 2016). Currently, the commercial quality of the Moroccan loquat genotypes is rare and the distinction between most trees is based on the shape of the fruit. In this context, this investigation aims to quantify the main bioactive compounds and to assess the antioxidant capacity of the eight loquat fruits which are chosen according to some agronomic and economic criteria, such as the earliness and tardiness, shape, size, and colour of the fruits and good health of the tree.

Materials and methods

1.1. Plant material

In 2016, a prospection conducted in the regions of Zegzel, Takerboust, Tazaghine, and Taghsrout, belonging to the region of Berkane, allowed to collect eight genotypes of loquat. The code assigned and the geographical origin of each genotype are shown in Figure. 1 and Table 1. The choice of these genotypes is based on size, fruit colour, agronomic characteristics and denominations. Indeed, the mature and healthy fruits were manually pitted, frozen in liquid nitrogen, and stored at -20°C for analysis.

1.2.Chemicals

All solvents (methanol, chloroform, ethanol, acetone, acetonitrile), Phenol, sulphuric acid (H_2SO_4), glucose, extraction buffer, Bradford reagent, phenolindo- 2,6-dichlorophenol (DPIP), sodium carbonate (Na_2CO_3), β-carotene, Folin–Ciocalteu reagent, gallic acid, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), rutin, vanillin, catechin, ascorbic acid, DPPH, ABTS, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), standards of organic acids and sugars. All other products used have an analytical grade.

1.3. Fruit weight measurement

The weight of 10 loquat fruits from each genotype was measured using an electronic balance (Precisa XB 2200 C, Precisa, UK).

1.4. Biochemical analysis

1.4.1. Profile of Sugar

In order to analyse the sugar profile, the protocol described by Chen *et al.* (2002) was applied. Soluble sugars were extracted by grinding 5g of frozen fruit in 5 volumes (w/v) of 12: 5: 3 (v/v/v) methanol/chloroform/water. The extracts were centrifuged at 5000 ×*g* for 5 minutes. The extraction was performed three times. Water and chloroform were then added to bring the final methanol/chloroform/water ratio to 10: 6: 5 (v/v/v) and the chloroform layer was removed. The remaining aqueous alcohol phase was adjusted to pH 7 with NaOH (0.1 mol/l), dried under vacuum and redissolved in distilled water. Sugars dissolved in water were analysed by high-performance liquid chromatography (HPLC) (Hewlett-Packard series 1100; Hewlett-Packard, Wilmington, DE, USA). Sugars were isolated using a Supelco column (Supelcogel TM C-610H column 30 cm × 7.8 mm) and Supelguard (5 cm × 4.6 mm; Supelco, Inc., Bellefonte, PA, USA).

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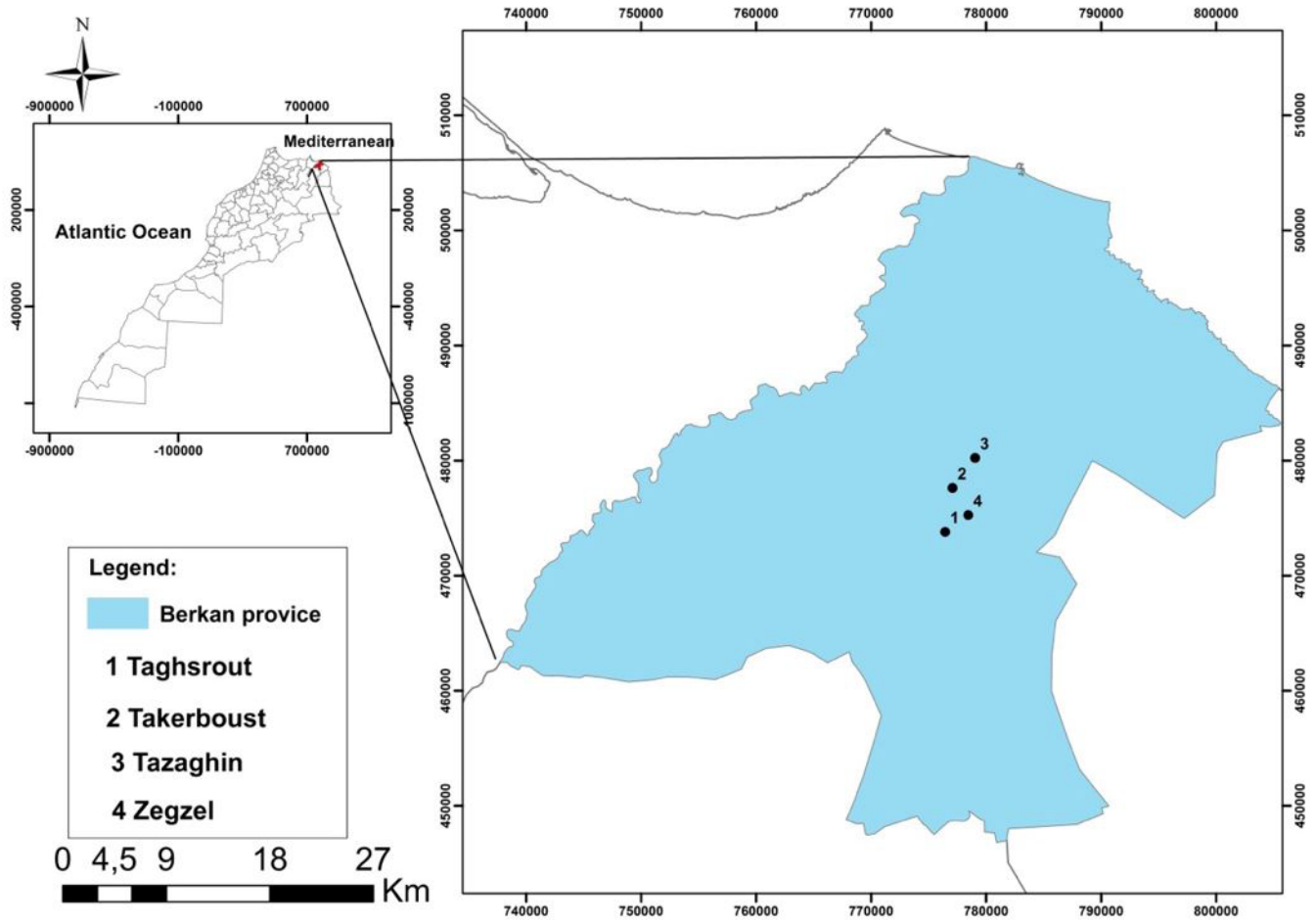

Figure 1. Location of sampling areas in North-Eastern Morocco

The column temperature was 90 ◦C and the elution buffer consisted of 0.1% phosphoric acid with a flow rate of 0.5ml mim-1. The absorbance was measured at 210nm using a refractive index detector. Standards of sugars (glucose, fructose, and sucrose) were obtained from Sigma (Poole, UK). Fructose, glucose, and sucrose were identified and quantified by comparison with retention times and integrated peak areas from external standards. The results were expressed as concentrations of mg per g of dry weight.

1.4.2. Profile of organic acids

Organic acids in the samples of loquat were quantified according to Hernández *et al.* (2016) protocol. Briefly, 0.5g of each sample lyophilized was subjected to ultra-sonication extraction for 30 min with 5ml of Milli-Q water and centrifugation at 15.000 ×*g* for 20min (Sigma 3–18 K; Sigma. Osterode am Harz. Germany). Then, one millilitre of the hydrophilic extract centrifuged was filtered through a 0.45 µm Millipore filter, and 10µl were injected into high-performance liquid chromatography (HPLC) (Hewlett-Packard series 1100; Hewlett-Packard, Wilmington, DE, USA). Organic acids were isolated using a Supelco column (Supelcogel TM C-610H column 30 cm × 7.8 mm) and Supelguard (5 cm × 4.6 mm; Supelco, Inc., Bellefonte, PA, USA). The elution buffer consisted of 0.1% phosphoric acid with a flow rate of 0.5ml mim-1. and absorbance was measured at 210nm using a diode-array detector (DAD). Standards of organic acids (citric, malic, quinic, and succinic) were obtained from Sigma (Poole, UK). Calibration curves were used for the quantification of organic acids, showing good linearity (R^2 = 0.999). Results for organic acids were expressed as concentrations mg /100g of dry weight.

1.4.3. Total proteins content analysis

The total protein assay is carried out according to the

method of Bradford, (1976. In fact, the extraction was carried out by mixing 1g of each lyophilized sample with 5 ml of buffer solution. Subsequently, 0.50 ml from the obtained filtrate was mixed with 2 ml of Bradford reagent. The tubes are incubated for 2 minutes at room temperature. Then the absorbance was read at 595 nm using a spectrophotometer (JASCO V-630). Bovine Serum Albumin (BSA) was used as a standard. The protein contents were expressed in g per 100g of fresh weight.

2.4.4. Vitamin C content

The vitamin C content was assessed in fruit juice using a 2,6-dichloroindophenol titrimetric method according to AOAC, (1995). 10 ml of sample were mixed with 40ml of buffer (1g/l oxalic acid plus 4 g/l anhydrous sodium acetate) and titrated against a colorant solution containing 295 mg/l DPIP and 100 mg sodium bicarbonate. A standard curve was generated using concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/l of ascorbic acid (BDH. Buffalo. NY. USA). Results were expressed as mg ascorbic acid equivalent (AAE) per 100g of fresh weight.

2.4.5. Carotenoid content

For the quantifying of carotenoid content, the method described by Reyes *et al.* (1999) was performed. Indeed, 2g of frozen fruit was homogenized with 25ml of acetone/ethanol (1: 1 v/v) containing 200mg/l butylated hydroxytoluene. The homogenate was filtered through #4 Whatman filter paper, washed in solvent (60 ml), and diluted to 100 ml with the extraction solvent. The extracts were placed in a plastic container with 50ml of hexane. After shaking the mixture was left to stand for 15 minutes before adding 25ml of nanopure water. The container was shaken a second time and the contents were allowed to separate for 30 minutes. Then, the absorbance was measured at 470 nm with a spectrophotometer (ASCO V-630). The carotenoid content was quantified using a standard curve of β-carotene (1–4µg/ml). Results were expressed as µg β-carotene equivalent per g of fresh weight.

2.4.6. Extract preparation for phenolic content and antioxidant capacity

The extraction was carried out according to the method described by Swain & Hillis (1959). Indeed, 10g of fruit were homogenized in 25 ml of absolute methanol using a waring blender. The homogenates were maintained at 4°C for 12 h, then centrifuged at 6000 rpm for 20 min and the supernatant is recovered. The extraction of the residue was repeated three times under the same conditions.

2.4.7. Total phenolic content

The Folin–Ciocalteu reagent assay was used to determine the total phenolic content (Singleton and Rossi, 1965). A 0.1ml aliquot of the extract was mixed with 5ml of Folin–Ciocalteu reagent (0.2 mol/l). The solution was allowed to stand at 25◦C for 5min before adding 4ml of sodium carbonate solution (150 g/l). The absorbance at 765 nm using a spectrophotometer (ASCO V-630) was measured after the initial mixing and subsequently for up to 90 min until it reached a plateau. Gallic acid was used as a standard for the calibration curve. The results were expressed as µg gallic acid equivalent (GAE) g^{-1} FW.

2.4.8. Total Flavonoids content

The total flavonoid content was measured using a colorimetric method developed by Zhishen J *et al.* 1999. Briefly, 2ml of the methanolic extract was mixed with 3ml of distilled water and 0.3ml of sodium nitrate (NaNO2, 0.72 mol/l). After 5 min, 0.6 of aluminium trichloride ($AlCl_3$) at 0.41 mol/l was added. Following 6 min, 2ml of sodium hydroxide (NaOH, 1M) and 2.1ml of distilled water were introduced. The absorbance is measured at 510 nm by using a spectrophotometer (ASCO V-630) and the results are expressed as µg of rutin equivalent (RE). g^{-1} FW.

2.4.9. Condensed tannin content

In the presence of concentrated H_2SO_4, condensed tannins are transformed into anthocyanidols due to their reaction with vanillin in the methanolic extract (Sun et al., 1998). To measure the carotenoid content, 50ml of methanolic extract was suitably diluted and mixed with 3ml of 4% methanolic vanillin solution and 1.5 ml of H2SO4. After 15 minutes, the absorbance was measured at 500nm using a spectrophotometer (ASCO V-630). Condensed tannins contents were expressed as µg of catechin equivalents per g ac-

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cording to the standard curve.

2.4.10. Antioxidant capacity analysis

DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl radical) radical scavenging activity of loquat extracts was measured according to the method of Braca *et al.* (2001). A quantity of 0.1 ml of fruit extract was added to 3ml of methanolic solution of DPPH (0.1mmol/l). After 30 min in dark, the absorbance at 517 nm was carried out with a spectrophotometer (ASCO V-630). The inhibitory activity (%) was calculated as:

$$\text{Percentage Inhibition (\%)} = \frac{Ab_{593\ \text{sample}}\ \text{nm} - Ab_{593\text{controle}}\ \text{nm}}{Ab_{593\ \text{sample}}\ \text{nm}} \times 100$$

Where:

Ab517 control: control absorbance.

Ab517sample: control sample absorbance.

The Results were expressed as µmol Trolox equivalent. g-1 FW.

ABTS assay

The ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical cation) radical scavenging ability of extracts was determined applying the method described by Re *et al.* (1999). ABTS was produced by reacting an ABTS solution (7 mmol/l) with Potassium persulfate (K2S2O8) (2.45 mmol/l) in the dark for 16 h. The absorbance was adjusted at 734 nm to 0.700 with ethanol. Then 0.2 ml of the appropriate dilution of the extract was added to 2ml of ABTS solution. The absorbance was measured at 734 nm after 15 min with the use of a spectrophotometer (ASCO V-630). The inhibitory activity (%) was calculated as:

$$\text{Percentage Inhibition (\%)} = \frac{Ab_{734\ \text{control}}\ \text{nm} - Ab_{734\ \text{sample}}\ \text{nm}}{Ab_{734\text{control}}\ \text{nm}} \times 100$$

Where:

Ab734 control: control absorbance.

Ab734 sample: control sample absorbance.

Results were expressed as µmol Trolox equivalent g^{-1} FW.

FRAP assay

With slight modifications, the FRAP test described by Benzie & Strain, 1996, was applied. The stock solutions included 300 mmol/l acetate buffer (3.1g of $C_2H_3NaO_2 \cdot 3H_2O$ and 16ml of C_2H_4O2) at pH 3.6, 10 mmol/l TPTZ solution in 40 mmol/l HCl and 20 mmol/l $FeCl_3 \cdot 6H_2O$ solution. A fresh working solution was prepared by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution, and 2.5 ml of FeCl3· 6H2O solution and then warmed at 37 ◦C before use. Next, 0.15 ml of fruit extract or methanol (reagent blank) was mixed with 2.85 ml of FRAP solution at 37 ◦C for 30 min in the dark (in a water bath). The absorbance was conducted at 593 nm using a spectrophotometer (ASCO V-630). Results were expressed as µmol Trolox equivalents g^{-1} FW.

$$\text{Inhibition (\%)} = \frac{Ab_{517\ \text{control}}\ \text{nm} - Ab_{517\ \text{sample}}\ \text{nm}}{Ab_{517\ \text{control}}\ \text{nm}} \times 100$$

Where:

Ab593 control: control absorbance.

Ab593 sample: control sample absorbance.

Results were expressed as µmol Trolox equivalent g-1 FW

2.5. Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) to indicate the signification differences using the Duncan Post Hoc at the 5% level. In addition, the correlation between studied parameters was analysed using the Pearson correlation coefficient ($\alpha = 0.05$). All analyses were performed using SPSS Ver. 28. software with three replicates for each parameter. Results were reported as mean ± SD.

3. Results

The present study revealed a large variation in fruit weight and biochemical content among eight loquat fruit genotypes.

3.1. Fruit weight

The most important commercial criterion for loquats is that of fruit weight. In the present study, with 10 fruits being analysed for each loquat genotype, the highest fruit weight (54.55 g) had been recorded for the Z1 genotype, while the TA6 genotype produced the smallest fruits (28.72 g) (table 1).

3.2. Total phenolic, flavonoid, carotenoid, vitamin C, protein, and tannin contents

The total phenolic compounds, flavonoids, carotenoids, condensed tannins, and protein as well as vitamin C contents of the eight genotypes are summarised in Table 2. Phenolic contents among the eight loquat fruit genotypes varied considerably, with the TZN1 and T2 genotypes showing the highest (186.05µg GAE g^{-1}) and lowest phenolic contents (92.22µg GAEg^{-1}), respectively. Concerning total flavonoids, maximum levels were detected for fruits belonging to genotypes T2 (59.58 µg RE g^{-1}) and Z1 (59.00 µg RE g^{-1}), whereas minimum levels were detected for genotype TZN1 (57.72 µg RE g^{-1}). Carotenoid contents, oscillated from 15.94 µg ß-carotene g^{-1} (Z7) to 90.65 µg ß-carotene g^{-1} (TA7). Regarding vitamin C, the highest and lowest levels had been detected in genotypes T2 (14.19 mg AAE/100 g) and TA7 (6.44 mg AAE/100 g), respectively. The protein and tannin contents exhibited no significant differences between genotypes with values of 0.64-0.98 g/100 g and 2.94-2.61 µg. g^{-1} respectively.

Table 1. Loquat genotypes used in this study

Locality	Code
Taghsrout2	T2
TakerboustA	TA5, TA6. TA7
TazaghinZ	TZN1. TZN2
Zegzel	Z1. Z7.

Table 2. Bioactive compounds of eight loquat genotypes

Genotypes	Weight fruit (g)	Total phenolic (µg GAE.g^{-1})	Total flavonoids (µg RE g^{-1})	Total carotenoids (µg ß-carotene g-1)	Tannins µg. g^{-1}	Protein g/100g	Vitamin C (mg AAE/ 100g)
T2	52.35^{c}	**92.22±0.10^{a}**	**59.58±0.10^{e}**	78.73±0.00^{f}	2.89±0.03^{a}	0.66±0.05^{a}	**14.19±0.98^{e}**
TA5	41 .67^{b}	150.93±0.16^{b}	58.59±0.01^{cd}	65.54±0.00^{e}	2.75±0.17^{a}	0.76±0.23^{a}	11.57±0.33^{d}
TA6	**28.72^{a}**	150.39±0.35^{b}	58.92±0.03^{d}	47.45±0.01^{d}	2.74±0.05^{a}	0.78±0.26^{a}	10.03±0.74^{bc}
TA7	29.95^{a}	176.79±0.49^{e}	58.41±0.06^{bc}	**90.65±0.00^{h}**	2.67±0.15^{a}	**0.64±0.08^{a}**	**6.44±0.33^{a}**
TZN1	42.09^{b}	**186.05±0.24^{f}**	**57.72±0.07^{a}**	80.13±0.01^{g}	**2.94±0.32^{a}**	0.89±0.28^{a}	8.85±0.95^{b}
TZN2	47.12^{bc}	169.36±0.16^{d}	57.86±0.07^{a}	49.94±0.01^{d}	2.89±0.06^{a}	0.82±0.27^{a}	10.42±0.28^{cd}
Z1	**54.55^{c}**	164.51±0.16^{c}	59.00±0.34^{d}	39.87±0.05^{b}	2.83±0.11^{a}	0.97±0.17^{a}	9.13±0.35^{bc}
Z7	41.19^{b}	169.36±0.15^{d}	58.07±0.17^{ab}	**15.94±0.00^{a}**	**2.61±0.04^{a}**	**0.98±0.30^{a}**	9.06±0.07^{bc}

Value in average ± SD. Values marked by the same letter, are not significantly different ($p < 0.05$) using Duncan post hoc test ($p < 0.05$).

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3.3. Sugar content

Genotype sugar content varied with a range of 54.85–28.03 mg. g^{-1} (Table 3). These amounts had been observed in TA6 and Z1 genotypes, respectively. The loquat fruits revealed high concentrations of fructose, followed by sucrose, and lower concentrations of glucose. The highest levels of fructose (TA5: 31.64 mg. g^{-1}), sucrose (TA6: 21.09 mg. g^{-1}), and glucose (TA7: 10.34 mg/100 g) had been observed in TA genotypes, while the TZN1 genotype showed the lowest levels for all three sugars (16.15 mg/100 g, 5.61 mg/100 g, and 6.27 mg/100 g respectively) (Table 3).

3.4. Organic acid contents

Organic acid profiles of the eight loquat fruit genotypes are presented in Table 4. The total amount of organic acids exhibited a large variation from 168.09 mg/100 g (TZN1) to 326.24 mg/100 g (T2). In addition, the HPLC analyses revealed high variation in the levels of four organic acids, with malic acid (100.74–193.75 mg/100 g) being the most prevalent, followed by quinic (51.28–107.25 mg/100 g), citric (12.64–15.36 mg/100 g) and succinic acids (0.67–12.6 mg/100 g). The T2 genotype showed the greatest quantity of malic, quinic, and succinic acids while the TZN1containted the highest level of citric acid.

3.5. Antioxidant activity

The antioxidant capacity of the different fruit genotypes, as determined by three different methods, is shown in Table 5. According to DPPH assays, the Z7 and TA7 genotypes showed the highest (53.94 63 µmol TE. g^{-1}) and lowest (12.63 63 µmol TE. g^{-1}) antioxidant activities, respectively. Similarly, the ABTS method indicated high antioxidant activity for Z7 fruits (58 63 µmol TE. g^{-1}), while the lowest activity had been recorded for TZN1 fruits (27.63 63 µmol TE. g^{-1}). Readings from the FRAP method ranged from 16.06 µmol TE /g for the TZN2 genotype to 130 µmol TE /g for the Z7 genotype. All three methods thus reported higher antioxidant levels for the Z7 genotype compared to that of the other loquat fruit genotypes.

3.6. Correlations between analysed parameters

Correlations among all biochemical parameters are summarised in Table 6. For the DPPH assay, a strong and positive correlation with polyphenols (r^2 = 0.75), flavonoids (r^2 = 0. 72) and condensed tannins (r^2 = 0. 72) had been revealed. In contrast, no correlation between vitamin C and the DPPH assay could be detected. Furthermore, total phenolics showed a strong correlation with flavonoids (r^2 = 0.671), while carotenoid content positively correlated with flavonoids (r^2 = 0.31). The other parameters did not reveal significant correlations.

4. Discussion

Weight and biochemical analyses of the eight fruit genotypes revealed a great biochemical richness of the Moroccan loquat. The fruit weights recorded in the present study (54.55–28.72 g) were similar to the finding of Durgac et al., (2006) (22.55–29.54 g), higher than those reported by Xu & Chen, (2010) and Zhang *et al.* (2015) (19.1-39.3g and 24.24-42.19g respectively), but lower than fruit weight revealed in Italian loquat, ranging from 38.4 to 74.2 g (Insero et al., 2003).

The assessment of the biochemical composition of the fruit is a very important criterion, especially for the identification of nutritional value and the antioxidant effect, which is due mostly to the phenolic compounds (Mansouri et al., 2005). The Moroccan loquat showed phenolic content levels of 92.22– 186.05µg GAE g^{-1} which is weak in comparison to that showed by Zhang *et al.* (2015) (9.90–13.73 mg GAE.g^{-1}) and Xu & Chen, (2011) (204.5–572.3µg GAE.g^{-1}). However, the total flavonoid levels (57.72–59.58 µg RE g^{-1}) had been similar to previous results obtained by Xu & Chen, (2011) (21.2-77.5 µg RE. g^{-1}). In contrast, fruit carotenoid contents (5.94– 90.65µg ß-carotene g^{-1}) were lower than the values reported by Xu & Chen, (2011), for Chinese loquats (23.4–496.3 µg β-carotene g^{-1}). Moreover, vitamin C levels noted in the present study (6.44–14.19mg AAE /100 g) had been more important than those recorded by Hasegawa et al., (2010) in Brazilian loquat (5.28–8.20mg/100 g) and also by Xu & Chen, (2011) in Chinese loquat (19.2–10.3 µg AAE g^{-1}) cultivars.

Sugar content is an important quality characteristic of fresh loquat fruits (Xu & Chen, 2011). Furthermore, the reducing sugars glucose and fructose together

Table 3. Sugar content of eight loquat genotypes (mg. g^{-1})

Genotype	Sucrose	Glucose	Fructose	Total sugar
T2	8.47±0.18^{b}	7.15±0.98^{ab}	29.01±0.52^{d}	44.63±0.33^{b}
TZN1	**5.61**±0.52^{a}	**6.27**±0.17^{a}	**16.15**±0.31^{a}	**28.03**±0.62^{a}
Z1	19.41±0.65^{f}	8.49±0.21^{cd}	25.34±0.01^{b}	53.24±0.27^{d}
TA7	16.47±0.72^{d}	**10.34**±0.03^{e}	27.18±0.41^{c}	53.99±0.42^{d}
TA5	10.79±0.35^{c}	8.76±0.75^{cd}	**31.64**±0.08^{e}	51.19±0.38^{c}
TA6	**21.09**±0.66^{g}	9.01±0.62^{cd}	24.75±0.66^{b}	**54.85**±1.03^{d}
Z7	17.72±0.52^{de}	9.51±0.38^{de}	24.36±0.47^{b}	51.59±0.55^{c}
TZN2	17.96±0.85^{e}	7.91±0.52^{bc}	25.35±0.33^{b}	51.22±0.11^{c}

Value in average ± SD. Values marked by the same letter, are not significantly different ($p < 0.05$) using Duncan post hoc test ($p < 0.05$). (Bold values are minimum and maximum)

Table 4. Organic acid composition of eight loquat genotypes in mg/100g DW

Genotype	Citric acid	Malic acid	Quinic acid	Succinic acid	Total
T2	**12.64**±0.27^{c}	**193.75**±0.58^{g}	**107.25**±0.25^{g}	**12.6**±0.25^{c}	**326.24**±0.33^{h}
TZN1	**15.36**±0.59^{e}	**100.74**±0.55^{a}	**51.28**±0.01^{a}	0.71±0.11^{a}	**168.09**±0.10^{a}
Z1	12.94±0.04^{cd}	160.13±0.83^{d}	74.12±0.33^{d}	**0.67**±0.03^{a}	247.86±0.95^{c}
TA7	13.63±0.57^{d}	160.52±0.52^{d}	73.44±0.48^{cd}	11.6±0.21^{bc}	259.19±0.13^{e}
TA5	0.896±0.01^{a}	187.79±0.10^{f}	105.46±0.14^{f}	12.58±0.62^{c}	306.73±0.41^{g}
T6	0.95±0.04^{a}	152.85±0.13^{c}	73.34±0.35^{c}	0.88±0.10^{a}	228.02±0.16^{c}
Z7	11.7±0.51^{b}	140.8±0.62^{b}	63.06±0.17^{b}	0.76±0.01^{a}	216.32±0.18^{b}
TZN2	11.65±0.33^{b}	172.55±0.35^{e}	82.61±0.13^{e}	11.05±0.06^{b}	277.86±0.02^{f}

Value in average ± SD. Values marked by the same letter, are not significantly different ($p < 0.05$) using Duncan post hoc test ($p < 0.05$). (Bold values are minimum and maximum)

Table 5. Antioxidant activity of eight loquat genotype as µmol TE. g^{-1}

Genotypes	ABTS	DPPH	FRAP
TZN1	**27.63**±0.30^{a}	28.71±0.30^{c}	58.32±0.41^{d}
TZN2	41.62±0.41^{c}	33.31±0.03^{e}	**16.06**±0.41^{a}
T2	45.74±0.42^{d}	30.85±0.31^{g}	42.57±0.68^{b}
Z7	**58**±0.17^{e}	**53.94**±0.14^{d}	**130.21**±0.03^{h}
Z1	39.9±1.02^{c}	26.08±0.24^{b}	61.46±0.31^{e}
TA5	44.76±0.24^{d}	28.47±0.34^{c}	105.68±0.47^{f}
TA6	44.65±0.55^{d}	37.06±0.18^{f}	108.82±0.65^{g}
TA7	32.83±0.49^{b}	**12.63**±0.25^{a}	28±0.44^{b}

(Bold values are minimum and maximum)

Table 6. Person's correlation among bioactive compounds and antioxidant activity

	DPPH	Polyphenols	Flavonoides	Carotenoids	Tannins
DPPH	1.00				
Polyphenols	**0.759**	1.00			
Flavonoids	**0.723**	**0.671****	1.00		
Carotenoids	**0.222**	0.202	**0.312****	1.00	
Tannins	**0.720**	-0.040	-0.002	0.003	1.00
Vitamin C	*-0.03*	-0,091	-0,046	-0,054	*<.0001*

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed).

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with sucrose constitute the majority of the soluble solids (Nunes et al., 1995). The main sugar in the Moroccan loquats had been fructose (16.15– 31.64 mg. g^{-1}), followed by sucrose (5.61– 21.09 mg. g^{-1}) and glucose (6.27–10.34 mg. g^{-1}). These results are in agreement with the levels of sugar revealed by Hasegawa *et al.* (2010) in Brazilian loquat cultivars, which are published in a range of 0.89-1.82; 2.70-4.96 and 0.15-1.51 g/100g respectively. In Chinese loquat, Xu & Chen (2011) reported comparable values to the present results (fructose: 35.9- 54.2 mg. g^{-1}, sucrose: 1.59 -5.02 mg. g^{-1} and glucose:27.4-53.6 mg. g^{-1}). As well as sugar, organic acids play a crucial role in fruit taste and flavor, which are important indicators of fruit quality (Liu et al., 2016).

The organic acid levels of Moroccan loquats analysed in the present study are the following: malic with 100.74–193.75 mg/100 g, quinic with 51.28–107.25 mg/100 g, citric with 12.64–15.36 mg/100 g, and succinic with 0.67–12.6 mg/100 g. These findings are lower than the values revealed by Toker et al., (2012) in Turkish loquat (malic: 364.93–842.49 mg/100 g, citric: 6.23–14.76 mg/100 g, and succinic: 10.38–30.79 mg/100 g). Also, Hasegawa *et al.* (2010) showed a high value in Brazilian loquat cultivars (malic: 587.97–988.05 mg/100 g, citric: 31.68–150.14 mg/100 g, and succinic: 13.78–24.71 mg/100 g). Regarding antioxidant activity, it is generally advised to combine at least two tests to obtain an accurate view of a food's total antioxidant capacity (Perez-Jimenez et al., 2008).

In the present study, the antioxidant activity of eight loquat genotypes had been assessed with DPPH, ABTS, and FRAP methods which recorded levels of 12.63–53.94 µmol/g, 27.63–58µmol/g and 16,06–130µmol /g respectively. These findings were in agreement with previous results which revealed a DPPH antioxidant level of 30.34 µmol TE g^{-1} and ABTS antioxidant capacity of 30.54 µmol TE g^{-1} (Ahumada *et al.* 2017). Many reports have highlighted the potential antioxidant activity of phenolic compounds in fruits, vegetables, beverages, and grains (De Ancos et al., 2000; Zielinski et al., 2000). Yet, several studies have found that the antioxidant activity of plant extracts may not be limited to phenolics, but that activity may be due to other secondary metabolites such as volatile oils, carotenoids, and vitamins (Javanmardi et al., 2003; Chanwitheesuk et al., 2005).

Regarding the correlation analyses, the antioxidant activities results showed a significant and positive correlation of DPPH assay with polyphenols, flavonoids, and condensed tannins. These correlations were in agreement with the finding that revealed positive and significant correlations of DPPH with total phenolics ($r = 0.706$) and flavonoids ($r = 0.759$) (Xu & Chen, 2011). Furthermore, total phenolics showed a strong correlation with flavonoids ($r^2 = 0.671$), which is similar to the result detected by Xu & Chen, (2011) ($r = 0.924$, $P < 0.01$). Indeed, several studies have shown a strong positive correlation between total polyphenol content and total flavonoids (Ercisli *et al.* 2012). In addition, the results recorded a significant correlation between carotenoid content and flavonoids, while Xu & Chen, (2011) found that no correlation was observed between carotenoids and the variables studied. Similarly, Gardner *et al.* (2000) demonstrated that the contribution of carotenoids to antioxidant potential was negligible. The other parameters studied did not show a significant correlation.

This variation in biochemical composition of Moroccan loquat may be due to genotypes, age of the tree, ecological factors, cultivation techniques, harvest date, and analysis conditions (Amoros et al., 2003; Chen et al., 2009; Xu & Chen, 2011).

5. Conclusion

The present investigation showed that the amount of major bioactive compounds and antioxidant capacity vary significantly among the eight genotypes except for the tannins and protein contents. According to the level of biochemical parameters studied in loquat, these genotypes should be considered the as first genotypes to be manipulated for propagation. Thus, Moroccan loquat can be considered a good source of natural compounds with antioxidant activity that can be used in therapeutic and condiment functional foods.

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Conflict of interest

The authors declare no conflicts of interest.

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