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Bioactive compounds and physicochemical attributes of loquat fruits in Mexico



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Compuestos bioactivos y atributos fisicoquímicos de frutos de níspero en México

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ABSTRACT

Keywords:

Antioxidant capacity
Carotenoids
Eriobotrya japonica
Fruit quality
Loquat seedlings
Total sugar content






The loquat is a fruit found in some regional markets of Mexico, and information on its quality is limited. The physicochemical properties and antioxidant potential of loquat fruit pulp produced in the states of Mexico, Oaxaca, and Veracruz were evaluated. The fresh weight (fw) of the fruits was about 15 g. The acidity of the pulp showed variations between 0.60 and 0.93%. The Total soluble solids values were between 14.5 and 17.3 °Bx. The concentration of total phenols was 3.5 mg g_{dw}⁻¹, and the flavonoids represented 62% of the bioactive compounds. The content of carotenes was higher in fruits from the State of Mexico (75.4 μg EβC g_{dw}⁻¹) that achieved the largest fruit size. In the ABTS and FRAP assays, Veracruz fruits had the highest antioxidant capacity but the smaller fruit size. The total sugar mean content was 6.8% fw. In general, the loquat fruit was of commercial small size, and in the tropical conditions of Veracruz attained high phenolic contents.

RESUMEN

Palabras clave:

Capacidad antioxidante
Carotenoides
Eriobotrya japonica
Calidad del fruto
Plántulas de níspero
Contenido de azúcares totales

El níspero es un fruto que se encuentra en los mercados regionales de México, y la información sobre su calidad es limitada. Se evaluaron las propiedades fisicoquímicas y el potencial antioxidante de la pulpa de frutos de níspero producidos en los Estados de México, Oaxaca y Veracruz. El peso fresco (pf) de los frutos fue de alrededor de 15 g. La acidez de la pulpa presentó variaciones entre 0,60 y 0,93%. Los valores de sólidos solubles totales estuvieron entre 14,5 y 17,3 °Bx. La concentración de fenoles totales fue de 3,5 mg g_{dw}⁻¹, y los flavonoides representaron el 62% del total de compuestos bioactivos. El contenido de carotenoides fue mayor en frutos del Estado de México (75,4 μg EβC g_{dw}⁻¹). En los ensayos ABTS y FRAP, los frutos de Veracruz presentaron la mayor capacidad antioxidante. El contenido medio de azúcar total fue de 6,8% pf. En general, el tamaño de la fruta en términos comerciales fue pequeño, y los frutos de Veracruz alcanzaron el mayor contenido de compuestos fenólicos.

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The loquat (*Eriobotrya japonica* Lindl.), also known as the Japanese plum (Costa et al. 2022), is an evergreen tree belonging to the Rosaceae family, originally native to China (Shah et al. 2023). Its cultivation has extended to various regions including Brazil, India, Japan, and Turkey (Xu and Chen 2011; Costa et al. 2022). In Colombia, loquat trees can be found growing in public urban forests in Bogota (Escobedo et al. 2015) and Medellin (Vergara-Navarro et al. 2007). Across Europe, the fruit is commonly sold in regions markets, while loquat trees adorn home gardens in Valencia, Spain, and Perugia, Italy. In Portugal, it grows in the Algarve region. However, in New Zealand, the loquat has occasionally been considered an invasive species (Tennyson et al. 1997).

This fruit is typically enjoyed fresh and is classified as non-climacteric (Alos et al. 2017). It is also used for the preparation of home remedies, and the chemical composition of its pulp shows anticancer, antiinflammatory, hypoglycemic (Li et al. 2009), antiviral, and hypolipidemic activities (Sagar et al. 2020).

In Mexico, there are few commercial orchards and mainly it grows in home gardens in mild temperate zones and tropical zones. It is produced commercially in the states of Mexico and Oaxaca, but it is widely distributed in the state of Veracruz. Most of the loquat trees in Mexico were propagated by seeds, and there are no cultivars originated and registered in Mexico. The fruit harvest period is from the end of October to January.

Most of the loquats at maturity are yellow and not many trees produce fruit of orange color in México. The volatiles of fruit aroma, total soluble solids (TSS), and titratable acidity (TA), have been used to estimate the optimum maturity of loquat (Shah et al. 2023) but the external color of the fruit has been established as the main determining factor of the harvest season (Chávez-Reyes et al. 2013), and it is attractive at the time of marketing.

The loquat tree has been little studied in Mexico despite that it grows in various climates and soils. In Guatemala, loquat seedling trees have been selected to produce fruits with adequate fruit size and good flavor for supermarkets (Cruz-Castillo et al. 2006), while in Mexico the production is for local markets and self-consumption.

The loquat fruit contains sugars, organic acids, polyphenols, and carotenoids among other compounds (Ding et al. 2001). In the ripe fruit, most of the sugars are fructose, sucrose, glucose, and sorbitol (Xu et al. 2010). The malic acid represents the majority of the loquat organic acids (Famiani et al. 2015).

In Mexico, there are few studies on the phenolic compounds of loquat fruits (Chávez-Reyes et al. 2013). The phenolic compounds are considered the most important antioxidant components in the loquat fresh fruit, and together with the fruit size, color, firmness, sugar content, and organic acids determine its quality parameters (Xu et al. 2014). The phenolic profile and the fruit quality of loquat are influenced by genetic and environmental aspects (Zhou et al. 2011). In Mexico, the evaluation of physicochemical, morphological, and biochemical properties of loquat covering several regions has not been studied.

This study aimed to investigate the morphology, physicochemical properties, and nutraceutical potential of loquat fruits cultivated in three distinct states of Mexico.

MATERIALS AND METHODS

Selection of trees

The loquat fruits were collected from seedling trees of about 20 years old, randomly selected in three states of the Mexican Republic in the communities of San Agustín Etla, Oaxaca (mild temperate zone); Acultzingo, and Ixhuatlan del Café in Veracruz (tropical zone); and Temascaltepec de González and Coatepec Harinas, State of Mexico (mild temperate zone). The harvest time of mature fruits between October and November 2020 was determined by the yellowish color of the peel (Figure 1).

Morphological characterization of fruits

The polar (PD) and equatorial (ED) diameters were measured with a digital vernier caliper (Mitutoyo model CD-6"CSX). The weight of fresh fruits (FW), skin (FS), pulp (FP), and dry weight of seeds (SW) were recorded using an OHAUS® CS200 digital scale. In addition, the number of seeds per fruit was quantified. Subsequently, the pulp/fruit ratio (FP/FW) was calculated. In total, 960 fruits were evaluated considering 320 fruits in each of the three states of the Mexican Republic.



Figure 1. Loquat fruits were harvested showing a yellow color on their skin.

Physicochemical analysis of the pulp

The total soluble solids of the loquat juice were measured using an Atago thermocompensated refractometer (AOAC 1990). Measurements were made in triplicate and the results were expressed in °Bx. To measure the pH, and acidity, 1 g of the fresh pulp was mixed with distilled water (10 mL) and extracted by vortexing (1 min, 3,000 rpm, in a Vortex synergy, WVR International), sonication was performed by 15 min with an Ultrasonic Cleaner 8890, Cole Parmer®, and incubation in a Prendo® INO-650 M Orbital Incubator by 30 min, at 30 °C. The mixture was centrifuged (15 min, 4,000 rpm, SOLBAT® J-600, Mexico) and the supernatant was transferred to a 10 mL volumetric flask and made up to volume with distilled water. The pH was measured from the extract using a digital potentiometer. The acidity was determined in a 5 mL aliquot of the extract, titrating with 0.01 N NaOH, until reaching a pH=8.0 (AOAC 1990). The acidity results were expressed in percentage (%) of malic acid (Famiani et al. 2015).

Antioxidant properties

The content of total phenols and flavonoids, as well as the antioxidant capacity of the loquat fruit pulp, were determined in extracts. Lyophilized and powdered pulp (0.6 g) were mixed with 80% methanol (25 mL) and extracted by vortexing (3 min, at 3,000 rpm, Vortex synergy, WVR International). Then, after adjusting to pH=3±0.3, the mixture was sonicated (15 min, ultrasonic cleaner 8890, Cole Parmer), incubated (30 min, at 30 °C, Prendo

INO-650M Orbital Incubator), and centrifuged (15 min, 4,000 rpm, SOLBAT® J-600 centrifuge, Mexico). The supernatant was calibrated with 80% methanol to obtain a final volume of 25 mL. The extracts were prepared in triplicate and stored in amber bottles under refrigeration. For the analyses, 96-cell microplates were used, the extracts were evaluated in quadruplicate and the absorbances were measured in a multidetector microplate reader with Gen5 software (Biotek Instruments Inc. Winoosky, VT, USA).

The total phenolic content was determined with the Folin-Ciocalteu method (Singleton and Rossi 1965) adapted to microplates. The extract (25 µL) was mixed with distilled water (125 µL), Folin-Ciocalteu reagent (20 µL), and 20% sodium carbonate (30 µL). The reaction mixture was stirred and allowed to stand for 30 min in the absence of light. The calibration curve was prepared with gallic acid (2.5-29.5 µg mL⁻¹). Absorbances were measured at 760 nm.

For the total flavonoid content (mg g_{dw}⁻¹) (Kubola and Siriamompun 2011), loquat pulp extract (0.5 mL), distilled water (2.5 mL), and 5% NaNO₂ (0.15 mL) were mixed and allowed to settle in a falcon tube. AlCl₃·6H₂O (0.3 mL) and 5% NaOH (1 mL) were then added, and vortexed (3,000 rpm, 3 min). The calibration curve was prepared with catechin (5-29.5 µg mL⁻¹). In each cell of a microplate, 200 µL of the reaction mixture were added and the absorbances were measured at 510 nm.

The antioxidant capacity was evaluated by the ABTS and FRAP assays. For the ABTS test ($\mu\text{mol ET g}_{\text{dw}}^{-1}$) (Re et al. 1999) an aliquot of 20 μL of the extract was mixed with 180 μL of the ABTS^{•+} solution, and after 15 min of reaction the absorbance at a wavelength of 734 nm was measured, using ABTS^{•+} (200 μL) as a control. The calibration curve was prepared with trolox (4.99-59.93 μM).

The FRAP assay (Benzie and Strain 1996) was adapted to microplates. An aliquot of 20 μL of standard or sample were mixed with 180 μL of FRAP solution and 60 μL of distilled water. 260 μL of FRAP were used as a blank. The calibration curve was prepared with trolox (3.8-46 μM). Absorbances were measured at 595 nm.

Total sugars

The phenol-sulfuric acid method (Yue et al. 2022) was used. The lyophilized pulp (0.1 g) was diluted in distilled water (50 mL). It was shaken in a vortex (2,500 rpm, 3 min), centrifuged (3,500 rpm, 10 min) and the supernatant was calibrated to 10 mL with distilled water, later dilutions were made in the proportions 1:8, 1:10, 1:15 and 1:20 for the different samples. In glass tubes, the sample extract (300 μL), 5% phenol solution (300 μL) and concentrated sulfuric acid (1.5 mL) were mixed, the mixture was left to stand for 1 h. The calibration curve was prepared with glucose (15.2-75.3 $\mu\text{g mL}^{-1}$). For the analysis, 96-well microplates with lids were used, in each well 200 μL of the reaction mixture was added and the absorbances were measured at 490 nm (Rao and Pattabiraman 1990) to obtain the total sugars in % fresh weight, considering that the loquats had about 85% of humidity.

Carotenoids

Loquat pulp (0.1 g) was mixed with 10 mL of hexane-acetone 3:2 v/v. The samples were vortexed (3,000 rpm, 1 min) and subsequently incubated (9 min, 30 °C). Finally, the mixture was centrifuged (1277 xg, 15 min) and the supernatant was calibrated to 10 mL with the extraction mixture. The absorbance was measured at 450 nm in a spectrophotometer. The calibration curve was prepared with β -carotene in a concentration range of 0.5-4 $\mu\text{g mL}^{-1}$ (Ordoñez et al. 2009). The carotenes concentration was reported in $\mu\text{mol ET g}_{\text{dw}}^{-1}$.

Statistical analysis

The experiments were analyzed with a completely

randomized block design for the three Mexican regions. An ANOVA analysis of variance and comparison of means of treatments was applied in the morphology of fruits, physicochemical and phytochemical properties of the fruit pulp (Tukey $P<0.05$) using the statistical package Infostat version 2015.

RESULTS AND DISCUSSION

Fruit morphology

The PD and ED of the fruits were different for the three regions. The loquat fruit of the State of Mexico, achieved higher length and wide than those of Oaxaca and Veracruz ($P<0.05$). Morton (1987) observed similar fruit lengths to those found in this study, and Aslmoshtaghi and Shahsavari (2013) reported similar values for ED and FW. Higher morphological values in length and wide were found in loquats of Turkey (Okatan et al. 2022). The loquat orchards sampled in the state of Mexico originated from seeds of selected fruit with large fruit size may influenced the large loquat fruit size recorded in the State of Mexico.

In the fruit, the pulp was the main component compared with the skin and the seeds (Table 1). The fruits of Veracruz had lower FW, FP, FS and SW, compared to those of the State of Mexico ($P<0.05$). In contrast, Gentile et al. (2016), and Feng et al. (2007), reported higher values for loquat fruit weights and diameters in fruits from Mediterranean countries and China, respectively. There were significant differences ($P<0.05$) for the FP/FW ratio, and the fruits of Veracruz had the lowest percentages, while those of the State of Mexico, had the highest. Thus, the loquats from Veracruz had less pulp weight, and the seeds and skin attained a higher percentage in the fruits compared with fruits from the other two regions. In general, the FP/FW values are similar to those indicated by Lin et al. (1999). Ercisli et al. (2012), determined that the weight of the fruit pulp is always greater than the seed, representing FP/FW of 80% which is higher than the found in the present study (Table 1).

Loquat fruit size is considered important in the European market. Fruit with large sizes achieve better prices (Costa et al. 2022). According to the quality standards for loquat fruit of the Ministry of Agriculture, Fisheries and Food of Spain (MAPA 1990), the fruits of Oaxaca (14.6 g) and Mexico (19.8 g) were classified as small, and the fruits from Veracruz were inadequate for the

Table 1. Morphological measurements of loquat fruits in three states of the Mexican Republic.

State	PD (mm)	ED (mm)	FW (g)	FS(g)	FP(g)	SW(g)	FP/FW (%)
Oax	32.6±0.3 ^{bt}	27.4±0.2 ^b	14.6±0.4 ^b	1.9±0.05 ^b	10.1±0.3 ^b	2.5±0.07 ^b	66.9±0.4 ^b
Ver	26.1±0.4 ^c	21.9±0.3 ^c	8.6±0.4 ^c	1.3±0.05 ^c	5.6±0.3 ^c	1.8±0.08 ^c	64.0±0.4 ^c
Mex	35.1±0.3 ^a	30.5±0.2 ^a	19.8±0.3 ^a	2.6±0.04 ^a	14.0±0.3 ^a	3.2±0.06 ^a	70.2±0.3 ^a
HMSD	1.11	0.79	1.26	0.15	1.0	0.23	1.28

Values with the same letter within columns are statistically equal based on Tukey's test ($P<0.05$) ± standard deviation. Oax: Oaxaca; Ver: Veracruz; Mex: State of Mexico; PD: Polare diameter; ED: equatorial diameter; FW: Fruit weight; FS: Fruit skin; FP: Fruit pulp; SW: seed weight. HMSD: Honest minimum significant difference.

European market. In Turkey (Ozturk and Ozturk 2018), fruit of 16 g is also considered of small size. In Mexico, people are accustomed to consuming small-sized fruits.

The fruits studied were harvested from seedling trees without agronomic management. Practices such as foliar application of B (Ali et al. 2022), fruit thinning (Lin et al. 1999), branch scratching, application of growth regulators (Agustí et al. 2007), introduction of cultivars, and selection of seedling trees with large fruit size (Cruz-Castillo et al. 2006) could support the development of the loquat fruit for markets with better payment. The loquat is not considered a tropical fruit tree. The small fruit size of the loquat fruit from Veracruz may be related to climatic factors, all the trees studied were

under tropical conditions from 300 to 1,500 m altitude. The information about loquat fruit growth in the tropics is scarce.

Physicochemical properties

In general, loquat fruit has acidity values between the range of 0.3-0.6% (Dhiman et al. 2021), and the fruits from the Oaxaca State presented 0.6%. The other fruits evaluated had juice acidity between 0.8 and 0.9% (Table 2). The pH values of loquat were similar to those found by Ali et al. (2020). The TSS of the fruits from Veracruz and Oaxaca were similar and higher ($P<0.05$) than those from the State of Mexico. Similar values of TSS were reported by Hasegawa (2010), Ercisli et al. (2012), Xu et al. (2014), Xu and Chen (2011), and Toker et al. (2013). The total sugar contents

Table 2. Physicochemical measurements of loquat fruits from three different states of the Mexican Republic.

State	TSS (°Bx)	pH	Total sugars (% FW)	Acidity (%)
Oax	16.6±0.4 ^{at}	4.1±0.05 ^a	7.48±0.68 ^a	0.6±0.06 ^b
Ver	17.3±0.4 ^a	3.7±0.05 ^c	6.00±1.25 ^a	0.9±0.07 ^a
State of Mex	14.5±0.3 ^b	3.9±0.04 ^b	7.00±0.77 ^a	0.8±0.05 ^{ab}
HMSD	1.27	0.15	3.37	0.20

Values with the same letter within columns are statistically equal based on Tukey's test ($P<0.05$) ± standard deviation. HMSD: Honest minimum significant difference.

values obtained for fruit produced from seedling trees in Mexico were slightly less than those found by Hasegawa et al. (2010) in Brazilian cultivars.

Bioactive compounds and antioxidant capacity

The fruits from Veracruz and the State of Mexico presented higher total phenolic content (TP) ($P<0.05$). The flavonoids (TF) were higher in loquat fruits from Veracruz, and this compound represented more than 50% of the total phenolic content (Table 3). The highest values of the ABTS test corresponded to the fruits of Veracruz. The values

determined by FRAP were similar for the fruit in the three states of the Mexican Republic ($P<0.05$) (Table 3).

In general, values for the antioxidant capacity, total content of phenols, and flavonoids were similar to those obtained in China by Xu et al. (2014), and Xu and Chen (2011), and in fruits of a local market in Mexico City (Chávez-Reyes et al. 2013). The concentration of total phenolic compounds in this study were similar to that reported by Ercisli et al. (2012), and Chávez-Reyes et al. (2013). Rivas et al. (2020), showed that there is a positive correlation between the

total phenolic content, flavonoids and antioxidant capacity, therefore, these compounds contributed greatly to the antioxidant capacity. In the present study, a trend of greater

antioxidant capacity was observed as the phenolic content increased, especially in the fruits from Veracruz and the State of Mexico (Table 3).

Table 3. Content of total phenols (TP), total flavonoids (TF) and antioxidant capacity (FRAP and ABTS), total sugars, and total carotenoids in three states of Mexico.

State	TP (mg g _{dw} ⁻¹)	TF (mg g _{dw} ⁻¹)	ABTS (μmol ET g _{dw} ⁻¹)	FRAP (μmol ET g _{dw} ⁻¹)	Carotenoids (μg g _{dw} ⁻¹)
Oax	3.2±0.1 ^{bt}	2.1±0.1 ^b	24.5±0.9 ^b	20.2±1.1 ^a	49.5±6.5 ^{ab}
Ver	3.8±0.2 ^a	2.6±0.2 ^a	28.2±1.4 ^a	22.5±1.5 ^a	30.3±8.4 ^b
Mex	3.8±0.1 ^a	2.1±0.1 ^b	23.9±0.8 ^b	20.6±0.9 ^a	75.4±7.3 ^a
HMSD	0.4	0.3	3.5	4.1	28.3

Values with the same letter within columns are statistically equal based on Tukey's test ($P < 0.05$) ± standard deviation. HMSD: Honest minimum significant difference.

The variation in the phenolic content can be influenced by environmental factors (Zhou et al. 2011; Friedman et al. 2009; Arámbula et al. 2010). In the present study, fruits from Veracruz under tropical conditions had the smallest size, but higher concentrations of phytochemicals (Table 3). Thus, a breeding program to increase fruit size will allow better characteristics of fruits from Veracruz. Loquat trees are adapted to subtropical or mild temperate climates (Costa et al. 2022), and their growth in tropical conditions may provoke stress and an increase of phenolic compounds in the fruit (Bryant and Julkunen-Tiitto 1995; Gershenson 1984; Okatan et al. 2022).

Total carotene content (Table 3) was higher in the loquat fruits of the State of Mexico. Ercisli et al. (2012) and Ferreira et al. (2009) found similar values for total carotene content. In the loquat pulp the carotene responsible for the yellow and orange colors are β-carotene and β-cryptoxanthin (Ding 1998). The synthesis of carotene and its accumulation in the fruit is influenced by environmental factors (Costa et al. 2022) and occurs during fruit ripening. When the content of carotenes in the pulp of the loquat fruit increases, the acidity decreases, and the total soluble solids and the content of glucose, sucrose, fructose and sorbitol, abscisic acid, increase (González et al. 2003; Shah et al. 2023).

CONCLUSION

The loquat fruits harvested in the State of Mexico had the larger weight and dimensions but had medium size according to international commercial standards. This fruit also had low TSS. In contrast, the loquat fruits from

Veracruz that showed smallest size showed higher TSS and higher concentrations of phenolic compounds and antioxidant capacity. However, the content of carotenes was lower. Loquat fruits from Veracruz and the State of Mexico showed higher acidity. The concentration of flavonoids in the loquat fruits was higher than 50% of the total phenolic content in all the fruits evaluated. The fruit studied was harvested from seedling trees with a lack of agronomic management, then, the fruit quality can be improved in Mexico. This is the first study showing bioactive compounds of loquat fruits in three States of the Mexican Republic. The physicochemical and bioactive compounds of the loquats through these regions could support studies aiming to the breeding of loquat trees to produce adequate fruit size in the State of Mexico and Oaxaca and/or the development of nutraceutical products derived from loquat fruit from Veracruz where higher total phenols and flavonoids contents were determined.

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