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ORIGINAL PAPER

Anthocyanin and Oil Contents, Fatty Acids Profiles and Antioxidant Activity of Mexican Landrace Avocado Fruits

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Abstract

Mexican landrace avocados are naturally distributed mainly in high areas of central Mexico, where they have been produced and consumed since pre-Hispanic times. However, trees of these species are being replaced by improved varieties with greater global demand, and many species have been lost due to the destruction of their natural habitats. Many people in Mexico like to consume the pulp and peel of these fruits and have done so since pre-Hispanic times. This is because the peel of Mexican landrace avocados, unlike the peel of Hass avocados, is very thin and flavorful. The peel color may be bluish-purple or dark reddish due to the presence of anthocyanins, which are compounds with antioxidant activity. The objective of this study was to assess the oil and anthocyanin contents and to evaluate the antioxidant activity in fruits of 11 accessions collected from producing-regions of Mexico. The oil content was 16.2 to 32.3 g 100 g^{-1} in pulp, and the main unsaturated fatty acids were oleic, linoleic and palmitoleic acids, depending on the accession. The anthocyanin contents in peels ranged from 0.64 to 47 mg g^{-1} fresh weight. The highest antioxidant activity was found in the peel (53.3–307.3 mmol g^{-1} fresh weight). The results confirm that the pulp and peel of dark-peel Mexican landrace avocados could be important nutraceuticals for humans.

Keywords Anthocyanins · Antioxidant · MUFA · Oil · Peel · PUFA · SFA

Abbreviations

MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
MSD	Mass selective detector
A	Absorbance
MW	Molecular weight
DF	Dilution factor
ε	Molar absorption
DPPH	2,2-diphenyl-1-picrylhydrazyl
As	Absorbance sample
Ab	Absorbance blank
Ac	Absorbance control
ANOVA	Analysis of variance
TE	Trolox equivalent
f. w.	Fresh weight

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Introduction

The avocado is one of Mexico's most important crops sold at the national and international levels. Mexico is the largest producer, exporter and consumer worldwide. Avocado production benefits growers, traders, industrializers and consumers generating thousands of jobs in any production area of the world. Because Mexico is part of the center of origin of the species, Mexican avocados possess broad genetic diversity. The species *Persea americana* var. Drymifolia is the cultivated species known as the Mexican avocado or the landrace avocado. The Mexican landrace avocado has been

differentiated from other races by its thin skin and small fruit. A vast variety of shapes and sizes exist in the valley of Mexico, where many people like to consume the pulp and peel of these fruits together and have done so since pre-Hispanic times because the peel is very thin and flavorful, and anise scented leaves [1–5].

Mexican avocados contain great genetic variability with almost unlimited possibilities for utilization. High quality oil is extracted from the avocado seed. The wood of the tree is used for handcrafts, and the leaves are used in traditional medicine and in traditional foods as a condiment to enhance flavor. It is also used as rootstock and, in some cases, as valuable genetic sources for the production of new varieties. Some of these Mexican landrace avocados have provided pest and disease resistance genes for commercial avocado varieties [5–7].

The Hass avocado has a diverse fatty acid profile with high percentages of monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and saturated acids (SFAs) [8]. In commercial varieties of avocado, such as 'Hass' and others, phytochemical compounds have been identified in the pulp, such as carotenoids, phenolic compounds, phytosterols, and anthocyanins in the peel [9, 10]. Avocado phytochemical compounds are important for their ability to capture free radicals that cause oxidative stress in cell structures and thus provide a beneficial effect in the prevention of cardiovascular, circulatory, cancerous and neurological diseases [11, 12]; however, these compounds have not been studied in Mexican landrace avocados. The aim of this study was to analyze the oil content, select phytochemical compounds and the antioxidant activity of some Mexican accessions of landrace avocados.

Materials and Methods

Plant Material The Mexican landrace avocado (*Persea americana* var. Drymifolia) was obtained from orchards located in Axocopan in the municipality of Atlixco (named Axocopan 1, Axocopan 2, Atlixco 1, Atlixco 2, Atlixco 3, Atlixco 4 and Atlixco 5), which has an average temperature of 19.4 °C and an altitude of 1870 m. Other samples were obtained from Tlalixtlipa and Hueyapan in the municipality of Zacatlán, Puebla, Mexico (named Tlalixtlipa 1 and Tlalixtlipa 2, Hueyapan 1 and Hueyapan 2), which has an average temperature of 15.1 °C and an altitude of 2130 m. Fruits were harvested at physiological maturity and transported in refrigerated trucks (5–7 °C) to the laboratory.

Sample Preparation Fruits were sanitized by immersion in a water solution of sodium hypochlorite (200 ppm) during 2 min and stored at room temperature until ripe (ready for consumption).

Chemical Analysis and Antioxidant Activity The preparation of samples for these assessments consisted of freezing the peel with liquid nitrogen and lyophilizing the sample (LABCONCO Freezone 4.5, Freezer, Canada) for 24 h (vacuum: 0.014 millibars, collector temperature: -51 °C).

The oil content in the mesocarp was quantified according to a previously methodology with modifications [13]. Chloroform (30 mL) and methanol (30 mL) were added to the mesocarp samples (20 g); the mixture was stirred for 5 min and allowed to stand for 24 h until phase separation. Boron trifluoride (0.5 mL) was then added to the 100 μL oil sample. The mixture was placed in a water bath for 20 min at 60 °C, then 1 mL of distilled water +1 mL of hexane was added. The nonpolar phase (esterified fatty acids) was extracted, and anhydrous sodium sulfate was added to remove moisture residues. The samples were stored at −20 °C until use.

The fatty acid profile was determined using a gas chromatograph (Agilent Technologies® 7890a, USA) coupled to a mass spectrometer (Agilent Technologies® 5975C, USA). For this procedure, a column (Agilent HP-5 MS) 30 m long with a 250 μm internal diameter and a 0.25 μm film thickness was used. The injection volume was 1 μL. Helium was used as the carrier gas with a flow rate of 1.6 mL min^{-1}. The oven temperature was set at 100 °C, the detector temperature (MSD) was set at 250 °C, and the quadrupole temperature was set at 150 °C. From this profile, the percentages of PUFA, MUFA and SFA were estimated.

Anthocyanins were quantified by the pH differential spectrophotometric method [14]. Lyophilized peel (1 g) was added to 10 mL 80% (v:v) methanol and sonicated for 20 min in a sonicator (Cole-Parmer, model 08892–21, USA). It was kept in the dark at room temperature for 24 h. The sample was filtered, and the methanolic extract was obtained. Two pH-regulator systems were used: hydrochloric acid [potassium chloride pH 1.0 (0.025 M)] and acetic acid [sodium acetate pH 4.5 (0.4 M)]. A diluted sample (0.2 mL) was added to 1.8 mL of the corresponding buffer solution, and the absorbance was determined at 510 and 700 nm in a spectrophotometer (Thermo model Genesys 10-S, USA). The regulatory solutions were used as references. Final absorbance was calculated with the formula: $A = (A_{510nm} - A_{700nm})_{pH\ 1.0} - (A_{510nm} - A_{700nm})_{pH\ 4.5}$.

$$\text{Anthocyanins}\left(\text{mg } 100\ g^{-1}\right) = A \times MW \times DF \times 1000/\varepsilon \times 1$$

where A = Absorbance, MW = Molecular weight (449.2), DF = Dilution factor and ε (molar absorption) = 26,900.

The concentration of monomeric pigments in the extract was expressed as cyanidin-3-glucoside equivalents.

The antioxidant activity of the peel was evaluated by the DPPH method (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich) [15]. In a test tube, 2.9 mL of a 0.1 mM DPPH solution and 0.1 mL of the extract were added, mixed with a

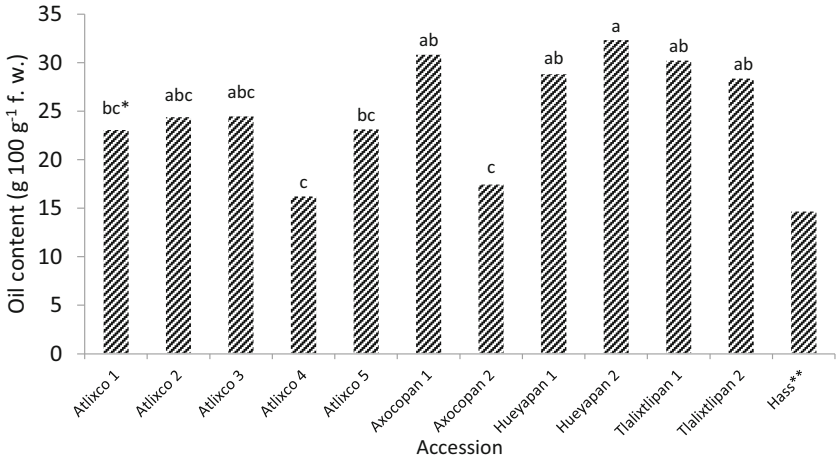

Fig. 1 Oil content in fruits of 11 accessions of ripe Mexican landrace avocados collected from different producing areas of Puebla State, Mexico, compared to 'Hass'.* Means with the same letter are statistically significant (Tukey's test; $p \leq 0.05$). **Reported by USDA (2004)

vortex mixer and incubated at room temperature in darkness until use. Absorbance readings were taken over 60 min at a wavelength of 516 nm in a Thermo® spectrophotometer (model Genesys 10-S, USA). Eighty percent methanol was used as a blank. Data were obtained according to the following formula:

$$\%DPPH_{Degraded} = \left[1 - \frac{As - Ab}{Ac - Ab}\right] \times 100$$

where As, Ab and Ac represent the absorbance of the sample, the blank and the control, respectively.

The results were reported as Trolox equivalents (mmol g^{-1} fresh weight).

Statistical Analysis Each Mexican landrace avocado accession was considered a treatment, and each fruit was a repetition. Each treatment or accession had 20 repetitions (fruits) for physical characterization and three repetitions for chemical characterization. ANOVA and a comparison of means (Tukey's test, $p = 0.05$) under a completely random design were performed. Pearson's correlation test was also performed for anthocyanins and antioxidant activity variables. MINITAB software (Minitab Inc., 2017) was used to perform all analyses.

Results and Discussion

Oil Content

The oil content of the mesocarp ranged from 16.20 to 32.32 g·100 g^{-1} f. w. among accessions (Fig. 1). In contrast, previous studies have reported an average oil content of 15.8 g·100 g^{-1} f. w. for the Hass cultivar [16]. It is clear that the oil contents in fruits of all the landrace accessions greatly surpassed those of the Hass variety. Furthermore, three accessions (Axocopan 1, Hueyapan 2 and Tlalixtlipa 1) had more than twice the oil content of Hass. For this reason, avocados from these accessions could be considered a good and natural source of high-quality oil for diverse uses.

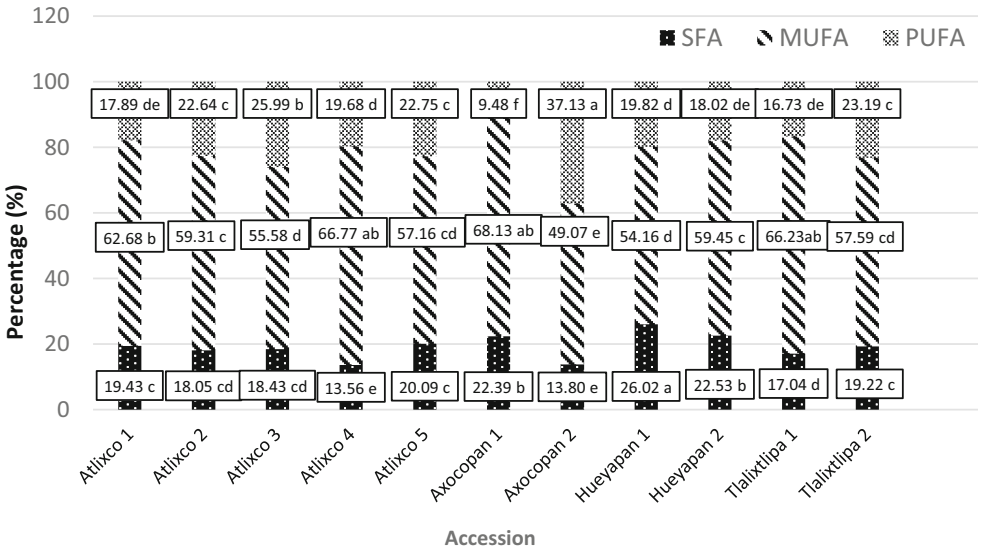

Fig. 2 Percent proportions of fatty acids (SFAs, MUFAs and PUFAs) in fruits of 11 accessions of ripe Mexican landrace avocados collected in Puebla State, Mexico. * Means with the same letter for each type of fatty acid are statistically significant (Tukey's test; $p \leq 0.05$)

Table 1 Percentage of the principal fatty acids in the oil extracted from the pulp of fruits of 11 accessions of ripe Mexican landrace avocados collected in Puebla State, Mexico

Accession	Oleic (%)	Palmitoleic (%)	Linoleic (%)	Palmitic (%)
Atlixco 1	55.95 ab*	6.73 c	9.11 a	19.43 bcd
Atlixco 2	48.26 de	6.39 c	7.87 abc	18.05 cd
Atlixco 3	48.77 de	6.8 c	8.74 ab	18.43 cd
Atlixco 4	58.5 a	3.36 d	9.26 a	13.56 d
Atlixco 5	48.9 de	5.96 cd	6.91 bcd	20.09 bcd
Axocopan 1	48.16 de	11.45 a	5.44 d	22.39 abc
Axocopan 2	43.1 ef	5.97 cd	7.41 abcd	13.8 d
Hueyapan 1	41.1 f	10.25 ab	7.91 abc	26.02 ab
Hueyapan 2	49.94 cd	9.51 ab	6.01 cd	22.53 abc
Tlalixtlipa 1	59.72 a	6.51 c	7.35 abcd	17.04 cd
Tlalixtlipa 2	51.15 bcd	6.44 c	6.42 bcd	19.22 cd

* Values with the same letter in a column are statistically significant (Tukey's test; $p \leq 0.05$)

Fatty Acids

The PUFA percent proportions ranged from 9.48 to 37.13% among accessions (Fig. 2); only Axocopan 1 had lower PUFA percent proportion than that reported in Hass avocados (13.62%) [6]. Fruits of the Axocopan 2 accession had the highest proportion of PUFA and the lowest proportion of SFA. It is notable that the proportion of PUFAs of these fruits was more than twice that reported for Hass. Atlixco 3 was also outstanding for its high proportion of PUFAs. These fatty acids, especially those of the omega-3 series, are precursors of anti-inflammatory lipid mediators [17]. For this and other reasons, a high dietary intake of PUFAs has been linked to a decreased risk of cardiovascular disease because they preserve high-density lipoprotein levels and act as antioxidants [18].

The MUFA percent proportions ranged from 49 to 68.13% among accessions. The highest percent proportions of MUFAs were found in fruits of accessions Axocopan 1, Atlixco 4 and Tlalixtlipa 1, whose values were near those reported for Hass avocado (71%) [19]. Moreover, the percentages of MUFAs were higher than those of PUFA percentages among accessions, while in six of the accessions, SFA proportions were lower than those of MUFAs and PUFAs. Fruits of accession Atlixco 4 also stand out for having the lowest content of SFA.

Oleic acid was found to be the main fatty acid in all accessions, representing 41 to 60% of total fatty acids (Table 1). slightly higher values have been reported for Hass avocados (67 to 70%) and for the Deuke cultivar (21 to 63%) [18, 20]. However, in our study, the oleic acid in Atlixco 1, Atlixco 4 and Tlalixtlipa 1 ranged from 55.9 to 59.8%, and these values are slightly higher than those reported for Hass avocados (51.2 to 54.8%) [21].

Palmitoleic acid ranged from 3.36 to 11.45% among accessions. These values were higher than those reported for Hass avocados (3.26%). However, only Axocopan 1 exceeded the palmitoleic acid percentage (10.7%) reported for Hass [18, 21].

Linoleic acid contents ranged from 5.44 to 9.26% among accessions. These percentages of this fatty acid were lower than the linoleic acid percentage reported for Hass avocados (13.30%) [6]. The accessions that had the lowest percentages of palmitic acid were Atlixco 4 and Axocopan 2.

Anthocyanin Content

A great variability (from 0.64 to 52.8 mg g^{-1} f. w.) in the anthocyanin content was found among the 11 studied accessions (Fig. 3). However, the values obtained for most of the accessions of the Mexican landrace avocados in this study

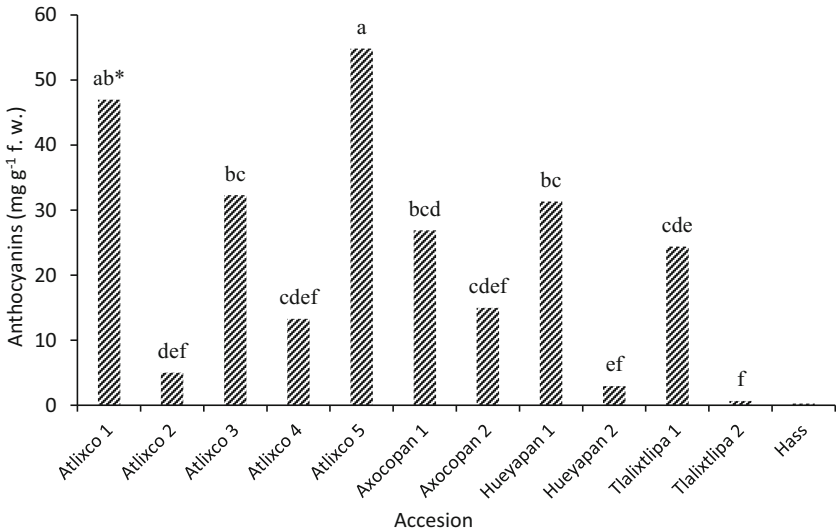

Fig. 3 Exocarp (peel) monomeric anthocyanins in fruits of 11 accessions of ripe Mexican landrace avocados collected in different producing areas of Puebla State, Mexico.* Values with the same letter are statistically significant (Tukey's test; $p \leq 0.05$)

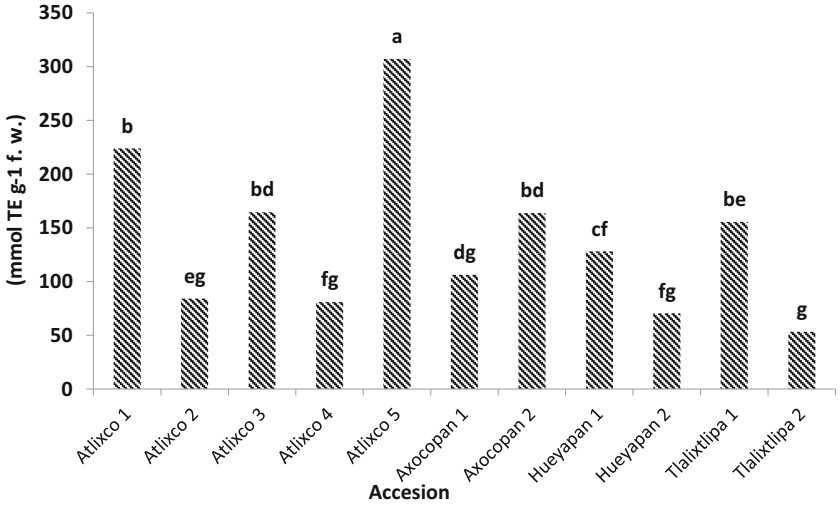

Fig. 4 Antioxidant activity (DPPH) of the exocarp (peel) in fruits of 11 accessions of ripe Mexican landrace avocados, collected from different producing areas of Puebla State, Mexico.* Values with the same letter in a column are statistically significant (Tukey's test; $p \leq 0.05$)

greatly exceeded the concentration of anthocyanins reported for Hass avocados (0.23 mg g^{-1} f. w.) [9, 22]. The fruit peel of the Atlixco 5 accession showed the highest anthocyanin content.

Antioxidant Activity in the Peel

The antioxidant activity in the peel of the 11 accessions studied ranged from 53.31 to 307.33 mmol TE g^{-1} f. w. However, most of the studied accessions showed values higher than 106 mmol TE g^{-1} f. w. The fruit peel of Atlixco 5 had the highest antioxidant activity. The fruit peel of Atlixco 1 also had high antioxidant activity, but this activity was significantly lower than that of Atlixco 5 (Fig. 4). Peels of Atlixco 5 and Atlixco 1 showed antioxidant activity values higher than those reported (165.10 ± 4.36 mmol 100^{-1} g f. w.) for the peel extracts of Hass avocados analyzed by the same method [23]. There are no previous reports of antioxidant activity of Mexican landrace avocado peel, probably because in the case of Hass and other commercial cultivars, avocado peel is normally not consumed by people. However, many Mexican consumers who live in the central part of Mexico like to consume the pulp and peel of these fruits and have done so since pre-Hispanic times because the peel of Mexican landrace avocados, unlike the peel of Hass avocados, is very thin and flavorful [2–5].

Conclusions

All the accessions had a very high oil content relative to that of Hass avocados. The predominant fatty acids in the oil content were MUFAs and PUFAs. The results indicated that both the pulp and peel of most of the studied accessions are important natural sources of oil, MUFAs, PUFAs and anthocyanins. In addition, because of the high antioxidant activity found in the fruit exocarp (peel) of most of the studied accessions, landrace avocados with a thin peel and dark colors (reddish, purple and black) should be consumed whole, without removing the peel, as it has been done by the Mexican people since pre-Hispanic times.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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