PHYSICAL AND CHEMICAL CHARACTERIZATION OF OIL THE FROM THE *Tapirira mexicana* Marchand SEED

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SUMMARY

In Mexican culture, seeds from endemic plants, such as the Tapirira mexicana *Marchand (locally known as* duraznillo*) are consumed. These seeds are eaten ripe, fresh or toasted; however, there is no information available about the physical characteristics and chemical composition of this seed. The purpose of this study was to determine the physical-chemical characteristics and fatty-acid profile of oil from* T. Mexicana *seeds. Seed dimensions and humidity were also determined. In the oil, obtained by pressing at 25°C, density, humidity, rancidity, color, saponification number, peroxides and acidity index were evaluated. Fatty acid profile was obtained through gas chromatography-mass spectrometry. Values of humidity, rancidity, peroxides and acidity index were below official standard levels. Linoleic acid concentration was 19 times higher than those found in* M. integrifolia. T. mexicana *oil has functional characteristics beneficial for human consumption, and could be used in the food industry. To our knowledge, this is the first report on the fatty acid composition of oil from the* T. mexicana *seed.*

Introduction

Fatty acids are fundamental nutrients in the human diet given the vital functions they perform in the body: they constitute the main energy reserve, transport a range of nutrients, regulate metabolism (Badui, 2012), are a structural part of cell membranes (Levant *et al*., 2004), and regulate several brain functions (Lee, 2013), among others.

As such, it is important to incorporate monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) into the diet, given that they can decrease the risk of developing cardiovascular diseases and help reduce total cholesterol, triglycerides and low-density lipoproteins, while increasing high-density lipoproteins (Vadivel *et al*., 2012). The current focus of food-related studies tends to be on products with high levels of MUFA and PUFA. As such, the USA Food and Drug Administration (FDA) has indicated that eating nuts is an important source of MUFA and essential fatty acids, and that the regular consumption of nuts has beneficial health effects (Alasalvar and Shahidi, 2009).

Furthermore, it is a common cultural practice among communities in Mexico to eat seeds from endemic plants, specifically the seeds of *Tapirira mexicana* Marchand, known locally as *duraznillo*, a native species to Mexico that is eaten in the states of Chiapas, Oaxaca, Puebla and Veracruz (Lascurain *et al*., 2010).

Seeds are picked by members of the local communities and sold in local markets, consumed ripe, fresh or toasted (using local, artisanal techniques) from July to September (Penington and Sarukhán, 2005). However, despite being a common practice in the region, to our knowledge no studies have been reported regarding the physical and chemical characteristics of the *duraznillo* seeds. The goal of this study was to evaluate the physical and chemical characteristics and the fatty acid profile of oil from *Tapirira mexicana* Marchand seeds.

Materials and Methods

Species

Tapirira mexicana Marchand is a genus of neotropical small plants trees belonging to the Anacardiaceae family; it is found in Mexico and parts of South America, mainly in Peru, Bolivia, Paraguay and Brazil (Wendt and Mitchell, 1995; Terrazas and Wendt, 1995a, b). The genus encompasses both trees and shrubs, with the tree reaching a height

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CARACTERIZACIÓN FÍSICA Y QUÍMICA DEL ACEITE DE LA SEMILLA DE *Tapirira mexicana* Marchand

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RESUMEN

*En México se consumen semillas de plantas endémicas como parte de la cultura. Ejemplo de esto es la semilla del duraznillo (*Tapirira mexicana *Marchand) cuyas semillas son consumidas maduras, frescas o tostadas. Sin embargo, no se tiene información disponible sobre las características físicas y composición química de esta semilla. El propósito de este estudio fue determinar las características físico-químicas y el perfil de ácidos grasos del aceite de semillas de* T. mexicana. *Las dimensiones y humedad de las semillas también fueron determinadas. En el aceite, obtenido por prensado a 25 °C, se evaluó densidad, humedad, rancidez, color, índice de saponificación, peróxidos e índice de acidez. El perfil de ácidos grasos se obtuvo mediante cromatografía de gases acoplado a espectrometría de masas. El porcentaje de humedad, densidad, peróxidos e índice de acidez se encontraron por debajo de lo establecido en las normas oficiales. Se identificaron los ácidos grasos en el aceite, encontrando al ácido linoleico 19 veces más elevado en comparación con el aceite de* M. integrifolia. *El aceite de la especie de* T. Mexicana *tiene características funcionales benéficas para el consumo humano por lo que podría utilizarse en la industria alimentaria. Hasta donde conocemos, este es el primer reporte sobre la composición de los ácidos grasos del aceite de la semilla de la especie* T. Mexicana.

CARACTERIZAÇÃO FÍSICA E QUÍMICA DO ÓLEO DE SEMENTE DE *Tapirira mexicana* Marchand

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RESUMO

No México se consomem sementes de plantas endêmicas como parte da cultura. Exemplo disto é a semente da Tapirirá (Tapirira mexicana Marchand) cujas sementes são consumidas maduras, frescas ou torradas. No entanto, não há informação disponível sobre as características físicas e composição química de esta semente. O propósito de este estudo foi determinar as características físico-químicas e o perfil de ácidos graxos do óleo de semente de T. mexicana. As dimensões e umidade das sementes também foram determinadas. No óleo, obtido por prensagem a 25 °C, se avaliou densidade, umidade, ranço, cor, índice de saponificação, peróxidos e índice de acidez. O perfil de ácidos graxos foi obtido mediante cromatografia de gases, acoplado a espectrometria de massas. A porcentagem de umidade, densidade, peróxidos e índice de acidez foram observados abaixo do estabelecido nas normas oficiais. Identificaram-se ácidos graxos no óleo, o ácido linoleico foi encontrado 19 vezes mais elevado em comparação com o óleo de M. integrifolia. O óleo da espécie de T. Mexicana tem características funcionais benéficas para o consumo humano por isto poderia utilizar-se na indústria alimentar. Até onde conhecemos, este é o primeiro relatório sobre a composição dos ácidos graxos do óleo da semente da espécie T. Mexicana.

of 30m, producing a wood similar to mahogany that is used in furniture. Its flowers are small, inflorescent and paniculate, and the fruit is fleshy and ovoid in shape, reaching 2cm in length (Penington and Sarukhán, 2005).

Sample collection

The fruits of *T. mexicana* were collected randomly from Rancho Plan de San Antonio, located on the high ground surrounding the town of Coatepec, Veracruz, Mexico (19°28'47.05''N, 96°59'28.1''W, altitude of 1,524masl). Average annual precipitation for the area is 1,035mm, and average annual temperature is 16-22°C.

Sample preparation

The fruits of *T. mexicana* were collected completely ripe and free of mechanical damage, and the pulp and shell were removed. The seeds were dried for 60 days at room temperature before removing the pericarp, prior to further analysis.

Physical characteristics of the seed

To measure the length and width of the seed and the thickness of the pericarp, a ±0.01mm digital vernier (Mitutoyo, mod. Absolute Digimatic, Japan) was used. The percentage of seed without shell and the humidity were calculated according to AOAC (1999).

Physical and chemical parameters of the oil

Oil extraction. The extraction of the oil was carried out by grinding 500g of shelled *duraznillo* seeds to obtain a paste, which was then compressed under a weight of 10t for 1min using a hydraulic press (PowerTeam, mod. A, USA). The oil obtained was centrifuged at 8000 rpm for 20min (Biofuge Primo R, Heraeus, USA) to eliminate any solid residue. The supernatant was placed in amber colored jars using nitrogen gas to eliminate any air. The oil was stored at -20°C for further analysis of total lipids content, rancidity, acidity index, saponification number, density, humidity, color parameters and identification and quantification of oil fatty acids.

The total lipids content was measured using the Soxhlet 930.39 method from AOAC (2000) using petroleum ether for extraction. Rancidity, acidity index, saponification number, density and humidity test were determined using the corresponding Mexican standard guidelines (NMX-F-222-SCFI-1995, NMX-F-075-SCFI-2006, NMX-F-174-SCFI-2006, NMX-F-211-SCFI-2006, NMX-F-101-SCFI-2012), while the peroxide number was determined in compliance with AOAC (2000).

The oil color was evaluated using a spectrophotometer (Konica Minolta, mod. CM-2500d). The parameters L*, a* and b* were used to calculate chromaticity ($C^* = (a^{*2}+b^{*2})^{1/2}$) and hue angle ($h° = \tan^{-1}(b^*/a^*)$) (McGuire, 1992).

Identification and quantification of fatty acids. Fatty acids were derived based on Hernández-Galán (2011), using chloroform and sodium methoxide. Fatty acid methyl esters (FAME) were analyzed using a gas chromatograph (Agilent mod. 7890A) and coupled to a mass detector (Agilent mod. 5975 C). The capillary column used was an HP-88 (Agilent) 100m×0.250mm×0.20µm. The conditions were described by Aquino-Bolaños *et al*. (2017). Helium was used as a carrier gas and mass spectra were obtained through electron-impact ionization at 70eV. Identification of the peaks for each fatty acid was carried out using MSD ChemStation E.02.00.493 software (Agilent) and the National Institute of Standards and Technology (NIST) database, and the identity of the most important fatty acids confirmed by the retention time and mass spectra of the corresponding methyl esters of fatty acids (Sigma-Aldrich). Quantification was achieved by using the relative area percentage method. All samples were analyzed in quadruplicate.

Statistical analysis

The comparison of the statistical significance of the average values obtained in the *duraznillo* seed oil with the benchmark values of the macadamia nut commonly consumed in the same region was done using the t-Student test. The statistical analysis was carried out using Minitab 17 software.

Results and Discussion

Physical parameters of the seed

The characterization of *Tapirira mexicana* Marchand seeds is shown in Table I. The seeds were found to be smaller than those reported for this species by Sugiyama and Peterson (2013) (13.3 ±0.13mm and 9.5 ±0.18mm vs 17.3 ±0.17 and 11.3 ±0.12mm, respectively). As to humidity, *T. mexicana* showed higher values (6.3 ±0.13%) compared to the *Macadamia integrifolia* nut, which has reported values of 3.48 ±0.27% (Mapel, 2014). When comparing the percentage of seed minus the shell it was observed that this level is similar to that reported for *M. integrifolia* (30.6 ±0.28% vs 30.31%, respectively) (Mapel, 2014).

TABLE I
CHARACTERIZATION OF *Tapirira mexicana* Marchand SPECIES SEED

Characteristics	
Humidity (%)	6.3 ±0.13
Seed without shell (%)	30.6 ±0.28
Pericarp thickness (mm)	2.3 ±0.07
Length (mm)	13.3 ±0.13
Width (mm)	9.5 ±0.18

Values expressed as averages ±SD.

TABLE II
PHYSICAL AND CHEMICAL PARAMETERS OF *Tapirira mexicana* Marchand OIL

Parameters	
Total lipids content (%)	73.1 ±0.17
Humidity (%)	0.17 ±0.02
Density (g/ml)	0.90 ±0.00
Peroxide value (mEq O2/kg)	2.2 ±0.05
Acidity index (mg KOH/g)	1.4 ±0.00
Saponification number (mg/g)	165.2 ±0.86
Rancidity	Negative

Values expressed as averages ±SD.

Physical and chemical parameters of the oil

Total lipids content of *T. mexicana* was 73.10g/100g dw (dry weight), a similar value to that in walnut (75.4g/100g; Li and Hu, 2011) and within the range reported by Aquino-Bolaños (2017) for different species and hybrids of macadamia (70.9 to 79.7g/100g). However, the value is higher than that of the fat content of nuts of common consumption, such as almonds (44.4-51.4g/100g; Moayedi *et al*., 2011), pistachios (53.9g/100; Li and Hu 2011) and peanuts (32.7-45.4%; Özcan, 2010).

The physical and chemical properties of *T. mexicana* seeds are shown in Table II. The percentage of humidity and volatile materials for *T. mexicana* oil was found to be of 0.17 ±0.02%, indicating that it has a low water content, which favors the stability (from oxidation) of the oil during storage. This value is higher than that reported for the oil from *M. integrifolia* nut, of 0.085% (Rodríguez *et al*., 2011).

The density of fatty acids and glycerides increases as its molecular weight decreases and its degree of unsaturation increases (Badui, 2012). The density of *T. mexicana* oil was found to be $0.90 g \cdot ml^{-1}$, lower than reported for the oil of different varieties of peanuts ($0.915\text{-}0.918 g \cdot ml^{-1}$; Shad *et al*., 2012) and M. integrifolia ($0.9116 g \cdot ml^{-1}$; Rodríguez *et al*., 2011).

Peroxides are the main products of primary autoxidative degradation. *T. mexicana* oil showed an average value (2.2 ±0.05mEq O_2/kg oil) below the standards of international regulations for crude, cold-pressed oil, the threshold of which is 15.0mEq O_2 active/kg oil (CODEX, 2009). The average value found is within the range reported by Kaijser *et al*. (2000) for the oil of different species of *Macadamia tetraphylla* (0.56-3.61mEq O_2/kg), but is higher than that reported for different species of almond (0.34-0.43mEq O_2/kg oil; Moayedi *et al*., 2011) and peanut (1.01 mEq O_2/kg oil; Özcan, 2010).

The acidity index of an oil indicates its free fatty acid content, so the lower the free fatty acid content, the better the oil quality is (Badui, 2012; Ibeto *et al*., 2012). *T. mexicana* oil has an acidity index of 1.4mg KOH/g oil (Table II), this value is in keeping with international regulations for crude cold-pressed oil, the threshold of which is 4mg KOH/g (CODEX, 2009). The value for *T. mexicana* oil is lower than that reported for oil from different varieties of peanut (3.96-4.95mg KOH/g oil; Shad *et al*., 2012), but higher than that reported for the oil of different species of almond (0.26-0.30mg KOH/g oil; Moayedi *et al*., 2011) and *M. integrifolia* (0.820mg KOH/g oil; Rodríguez *et al*., 2011).

The saponification number is expressed as the number of mg of KOH required to saponify 1g of the sample (Nayak and Patel, 2010). This value is inversely related to the average molecular weight of the fatty acids (Badui, 2012). In this study, the saponification number for *T. mexicana* oil was 165.2 ±0.86mg KOH/g oil, a similar value as that reported for peanuts (165.3-187.6mg KOH/g oil; Özcan, 2010). This number is higher than that for cashew kernel oil (137mg KOH/g oil; Akinhanmi *et al*., 2008) and lower than that for *M. integrifolia* (233.656mg KOH/g oil; Rodríguez *et al*., 2011). The low saponification number reported for *T. mexicana* is a sign that the oil is not appropriate for soap manufacturing. The rancidity test for *T. mexicana* was negative.

Evaluating the color of oil is necessary given that the appearance of the product is the main element that a consumer evaluates prior to purchasing it. In this study, *T. mexicana* oil is shown to have a clear, bright yellow tone with values of 11.4 ±1.32 for parameter b* and 2.7 ±0.32 for a*. The values for C* and hº, indicatives of chroma and hue, were 11.7 ±1.26 and 76.7 ±2.48, respectively (Table III). In general, it is preferable that edible oils be translucent and clear in color. The oil under study has characteristics that are desirable for consumers.

Fatty acid composition

Ten fatty acids were identified in *T. mexicana* seed oil, of which oleic, linoleic, palmitic and stearic acids were found in the greatest proportions, corresponding to 97.3% of the total fatty acids (Table IV).

Several studies have reported that saturated fatty acids, such as stearic acid (C18:0), have a positive impact on human health, given that they have antioxidant, anti-inflammatory (Nanji *et al*., 2001; Wang *et al*., 2007; Pin-Ho *et al*., 2010), hepatoprotective, decreasing hepatic lesions caused by cholesterol (Pin-Ho *et al*., 2010) and neuroprotective (Wang *et al*., 2007) effects, in addition to decreasing blood cholesterol levels (Ruddle *et al*., 2013). As such, when comparing the fatty acid profiles of *T. mexicana* and *M. integrifolia* (Aquino-Bolaños *et al*., 2017) nuts, it can be seen that palmitic (C16:0) and stearic (C18:0) fatty acids were found in greater concentrations in the *T. mexicana* seed (41% and 74%, respectively; Table IV).

The seed of *T. mexicana* contains a high percentage of monounsaturated fatty acids and polyunsaturated fatty acids (Table IV). The specific species studied in this research project contains a higher proportion of oleic fatty acids (C18:1), the consumption of which has been associated with a decreased risk of cardiovascular disease, arrhythmia and stroke, among others (Morón, 2008; Gil and Serra, 2013). Regarding linoleic acid (C18:2), levels in *T. mexicana* are 19 times higher when compared to *M. integrifolia*. This essential fatty acid helps metabolyze polyunsaturated fatty acids, is a necessary element of cutaneous fat and its consumption is associated with decreased coronary risk (Gil and Serra, 2013; Mataix and Gil, 2004).

It has been reported that the consumption of palmitoleic acid (C16:1) is associated with cardiac arrhythmia (Oyanagi *et al*., 2015) and mortality due to cardiovascular issues (Ebbesson *et al*., 2010). In this study, the amount of palmitoleic acid (C16:1) was 92% lower than to that reported for *M. integrifolia*. In terms of C18:3 (α-linoleic) content, there is no significant difference between *T. mexicana* and *M. integrifolia* (Table IV).

The MUFA/SFA index is used to evaluate the nutritional value and the healthy consumption of fat (Mamani-Linares and Gallo, 2013). According to the results obtained in this study, the amount of monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) found in the seed of *T. mexicana* are significantly different when compared to those reported for *M. integrifolia* (Table IV). The amount of polyunsaturated fatty acids (PUFA) is 17 times higher in *T. mexicana* seeds when compared to *M. integrifolia* (Table IV), which highlights that the *duraznillo* seed being studied could be used as an important source of fatty acids when included in the diet of the community.

TABLE III
COLOR PARAMETERS OF *Tapirira mexicana* Marchand OIL

Color parameter	
L*	29.8 ±1.02
a*	2.7 ±0.32
b*	11.4 ±1.32
C*	11.7 ±1.26
h°	76.7 ±2.48

Values expressed as averages ±SD.

TABLE IV
FATTY ACID PROFILE FROM *Tapirira mexicana* VS *Macadamia integrifolia* OIL

Fatty Acids	*T. mexicana*	*M. intergrifolia*	*p-value*
Palmitic, C16:0	18.5 ±0.18	13.1 ±1	0.00
Palmitoleic, C16:1	1.9 ±0.55	24.0 ±2	0.00
Stearic, C18:0	10.6 ±1.67	6.1 ±0.8	0.00
Oleic, C18:1	37.2 ±0.25	45.0 ±4.0	0.03
Linoleic, C18:2	31.0 ±0.62	1.6 ±1.6	0.00
α-Linolenic, C18:3	0.1 ±0.01	0.2 ±0.02	0.99
Arachidic, C20:0	0.6 ±0.02	3.9 ±0.4	0.00
Eicosenoic, C20:1	0.1 ±0.02	3.5 ±0.4	0.00
Behenic, C22:0	0.1 ±0.01	1.3 ±0.2	0.00
Lignoceric, C24:0	0.1 ±0.03	0.4 ±0.04	0.99
SFA	29.9 ±1.68	24.8 ±1.33	0.00
MUFA	39.1 ±0.60	72.5 ±4.39	0.00
PUFA	31.0 ±0.63	1.8 ±1.6	0.00
MUFA/SFA	1.3 ±0.07	2.9 ±0.24	0.00

Values are expressed as averages ±SD. The averages for *Macadamia integrifolia* were reported by Aquino-Bolaños *et al*. (2017). The statistical significance of the differences between the averages was evaluated using statistical evidence of t-student. Individual fatty acids were calculated as the percentage of total fatty acids. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

Conclusions

Oleic, linoleic, palmitic and stearic acids were identified in the *Tapirira mexicana* Marchand seed. The fatty acid profile indicates that *T. mexicana* seeds have functional benefits for human consumption. Furthermore, the extraction of the oil from this seed could be useful for industrial purposes, given the physical and chemical characteristics of the oil. According to our knowledge, this is the first report on the fatty acid composition of the *T. mexicana* Marchand seeds.

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