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Evaluation of acute toxicity and chemical composition of refined oil *Moringa oleifera* cultivated in Mexico

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Abstract

The oil obtained from *Moringa oleifera* seeds is mainly composed of oleic acid and in less proportion by linoleic and *α*-linolenic acids. It also contains phospholipids and other minority components, like enzymes, alkaloids, and glycosinolates some of which can generate undesirable characteristics and toxicity; therefore, refining processes are recommended for their removal. The aim of this work was to evaluate the effect of chemical refining on acute toxicity, fatty acid composition, and physicochemical properties, and of *M. oleifera* seed oil obtained from a Mexican variety. The oil was extracted by mechanical pressing of the seeds and then submitted to chemical refining. The crude and refined oils were characterized by determining the following parameters: acute toxicity in a murine model, fatty acid profile; iodine, saponification, and peroxide indexes; titratable acidity; and antioxidant capacity. Results showed that the *M. oleifera* seed oil did not present acute toxicity in the range of 300-2,000 mg/kg; therefore, could be used for human nutrition. The refining process did not have a significant effect ($p < 0.05$) on the content of oleic (69%), linoleic (0.74%), and *α*-linolenic (1.97%) acids. After the refining process, the iodine and saponification indexes increased. In contrast, the peroxide index, acidity, β-carotene content, and antioxidant capacity decreased.
Keywords: monounsaturated fatty acids, polyunsaturated fatty acids, refining process, *Moringa oleifera* seed oil, acute toxicity.

Evaluación de la toxicidad aguda y composición química de aceite refinado de *Moringa oleifera* cultivada en México

Resumen

El aceite de *Moringa oleifera* está compuesto principalmente de ácido oleico, linoleico y α-linolénico, también contiene fosfolípidos y otros componentes minoritarios, como enzimas, alcaloides y glucosinolatos, compuestos que pueden generar características no deseadas y/o toxicidad, sin embargo, éstos pueden eliminarse mediante un proceso de refinación. El objetivo de este trabajo fue evaluar el efecto de la refinación química sobre la toxicidad aguda, la composición de ácidos grasos, y las propiedades fisicoquímicas del aceite de semilla de *M. oleifera* de una variedad mexicana, para ésto, el aceite se extrajo por prensado mecánico de las semillas para someterse a refinación química. Al aceite crudo y refinado se les determinó toxicidad aguda probada en un modelo murino, así como también el perfil de los ácidos grasos, los índices de yodo, saponificación y peróxido, además de la acidez, y capacidad antioxidante. Los resultados mostraron que el aceite de semilla de *M. oleifera* no presentó toxicidad aguda en el intervalo de 300-2,000 mg/kg; por lo que podría ser utilizado para consumo humano. El proceso de refinación no tuvo efecto significativo ($p < 0.05$) sobre el contenido del ácido oleico (69%), linoleico (0.74%) y α-linolénico (1.97%). Después del proceso de refinación, aumentó el valor del índice de yodo y de saponificación, mientras que el índice de peróxido, la acidez, el contenido de β-caroteno y la capacidad antioxidante disminuyeron.
Palabras clave: *Moringa oleifera* aceite, ácidos grasos monoinsaturados, ácidos grasos poliinsaturados, proceso de refinación, toxicidad aguda.

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Introduction

The oil obtained from *Moringa oleifera* (*M. oleifera*) seeds is highly monounsaturated on account of its oleic acid content, which is higher than 60% (Chiou & Kalogeropoulos, 2017). The consumption of oleic acid reduces the plasmatic concentration of cholesterol, plays an important role in decreasing the incidence of brain disorders, such as dementia and Alzheimer´s, and inhibits the occurrence of colorectal cancer (Srivastava & Bhargava, 2012) In addition to oleic acid, *M. oleifera* seed oil contains linoleic acid (0.3-1.3%) and α-linolenic acid (0.3-0.5%) (Anwar, Zafar & Rashid, 2006; Sánchez-Machado *et al*., 2015). These fatty acids are essential and from them, other long-chain polyunsaturated fatty acids are synthetized in the organism. The latter give origin to eicosanoids (leukotrienes, prostaglandins, and thromboxanes), which act as hormonal cell messengers (Saini & Keum, 2018).

Eicosanoids synthetized from linoleic and α-linolenic acids have antagonistic effects in the human organism. Linoleic acid (ω6) derivatives generate inflammatory, thrombotic, and arrhythmic metabolites, which play an important role in the immune system. In contrast, linolenic acid (ω3) derivatives have an anti-inflammatory, anti-thrombotic, and anti-arrhythmic effect (Saini & Keum, 2018). For this reason, a specific ratio of these fatty acids (ω6:ω3) must be consumed. The recommended consumption ratio, in order to avoid a pro-inflammatory or immunodeficient state, is in the range of 1:1-2:1 (Orsavova, Misurcova, Vavra, Vicha & Mlcek, 2015).

Due to the health benefits that the consumption of oleic, linoleic, and α-linolenic acids provides, it is preferred to use vegetable oils containing these fatty acids, such as *M. oleifera* seed oil (Orsavova *et al*., 2015).

M. oleifera seeds and *M. oleifera* seed oil toxicity has been previously reported in some studies. Al-Said *et al*. (2012), and Ilesanmi**,** Gungula & Nadro (2017) reported that the *M. oleifera* seed oil, not presented acute toxicity in rats. While, Chivapat *et al*. (2012) reported the presence of acute toxicity signs and lethality, when administering an ethanolic extract of seeds to ICR mice, and Al-Anizi, Hellyer & Zhang (2014) reported that the cytotoxicity and genotoxicity of the hydrophobic extract of seeds, attributing such toxicity to alkaloids, enzymes, and glycosinolates associated to oil.

The concentration of the toxic compounds (like alkaloids) in *M. oleifera* seeds, depends on the geographical region and the climatic conditions in which the plant develops (Ukwueze, Okogwu, Ebem, Nwonumara & Nwodo, 2019). There has been no study performed on the toxicity of the oil obtained from *M. oleifera* seeds cultivated in Mexico.

The alkaloids, enzymes and glycosinolates associated with the *M. oleifera* oil toxicity, can be removed through a refining process (Siano *et al*., 2016), therefore this process could be an alternative to reduce the oil toxicity. However, in addition to the removal of toxic compounds of the oil, natural antioxidants are also lost during the refining process. Consequently, the oxidative stability of some oils is reduced after the refining process (Siano *et al*., 2016). Besides the loss of bioactive compounds during this process, harmful substances like chloropropanols (Zulkurnain *et al*., 2012) and trans fatty acids (Vaisali, Charanyaa, Belur & Regupathi, 2015) can be formed. Thus, there is a new tendency towards the consumption of vegetable oils without refining (crude oils) (Durmaz & Gökmen, 2019).

Therefore this work aimed to evaluate the effect of the refining process on the acute toxicity of *M. oleifera* seed oil obtained from a Mexican variety, the fatty acid profile, and the physicochemical properties of crude and refined oil.

Materials and methods

Moringa oleifera seeds

M. oleifera seeds were grown and purchased in Morelia, Michoacán, Mexico. The seeds were manually cleaned and peeled; and subsequently stored in plastic bags until the oil extraction.

Chemical reagents

All chemicals used in this work were reagent grade. Hexane, hydrochloric acid, ethanol, sodium hydroxide and potassium hydroxide were purchased from J.T Baker; acetone, and potassium iodide were purchased from Fermont; acetic acid and dichloromethane were purchase from Meyer; sodium thiosulfate was purchase from Hycel, iodine monobromide and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma Aldrich

Oil extraction

The oil was obtained by pressing with a hydraulic press (500, Taiwan) at a pressure of 500 kg_f/cm^2. During the extraction, temperature was set to 85 °C in order to inactivate enzymes from the oil, which could act as toxic components. The oil extraction yield was calculated with Equation 1.

$$\text{Oil extraction yield (\%)} = \left(\frac{Ap}{As}\right) \times 100 \qquad (1)$$

Where: Ap is the mass (g) of the oil of 100 g of seeds extracted by mechanical pressing; and As is the mass (g) of the total oil of 100 g of seeds.

Oil refining process

The chemical refining process was carried out following the methodology described by Crexi, Monte, Soares & Pinto

(2010), with some modifications. This process consisted of four stages: degumming, neutralization, washing, and bleaching. The degumming stage was performed by adding water to the oil in proportion 1:4, followed by the addition of 1% of concentrated hydrochloric acid, in relation to the oil mass. The mixture was heated at 80 °C for 30 min, continuously stirring. Then, the oil was centrifuged (Hermle Z326K, Wehingen, Germany) at 10,000 rpm for 15 min. The neutralization stage was carried out by heating the oil at 75 °C and adding 15% of sodium hydroxide 1 mol/L, in relation to the oil mass, and stirring the mixture for 15 min. Then, the oil was centrifuged at 10,000 rpm for 15 min. Subsequently, for the third stage of the refining process, the oil was washed three times with water at 90 °C. The bleaching stage was performed by adding activated carbon to the oil and finally centrifuging it at 10,000 rpm during 25 min. The refined oil recovery yield was calculated for each stage of the refining process using Equation 2.

$$\text{Refined oil recovery yield (\%)} = \left(\frac{P_0}{P_i}\right) \times 100 \qquad (2)$$

Where: P_o is the mass (g) of oil obtained at the end of each stage of the refining process and P_i is the initial mass (g) of oil.

Determination of acute toxicity

The acute toxicity of the crude and refined *M. oleifera* oils was evaluated following the guidelines of the Organization for Economic Cooperation and Development guidelines OECD No. 423 (2001) in male ICR mice, weighing between 0.031-0.038 kg. Before starting the trial, the mice were kept in laboratory conditions during seven days for an adaptation period. The mice were divided in three groups of three mice each. The oil was orally administered to the mice with a cannula. Two doses (300 and 2,000 mg/kg of body weight) of the crude oil were administered and a single dose of the refined oil (2,000 mg/kg). The overall behavior of the mice was continuously monitored during the first two hours after the oil administration; then, every 2 h during 24 h, and finally, daily during 14 days, recording toxicity signals (piloerection, tremors, convulsions, diarrhea, and/or lethargy) and mortality. The animals were weighed before the administration of the oil and at the end of the trial. Finally, the mice were sacrificed by dislocation, removing the heart, liver, kidneys, and spleen for observation and weighing.

Fatty acids profile

The fatty acids profile of *M. oleifera* crude and refined oils for the saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids was determined by gas chromatography, following the methodology described by the AOCS Ce 1h-05 (2005). A gas chromatographer (Agilent 7890 B, Mexico City, Mexico) equipped with a flame ionization detector (FID), an HP-88 column (100m*0.25m*0.20µm, Agilent, USA), and a data acquisition software (Chemstation version B.04.01.) was used for the determination. For the temperature program, the initial oven temperature (180 °C) was held for 10 min, then, increased to 220 °C at a rate of 3 °C/min, and finally held at 220 °C during 17 min. Helium was used as the carrier gas at a flow rate of 2 mL/min with an injection volume of 1 µL. The qualitative composition of the fatty acids was determined by comparing the retention times of the peaks obtained for the sample with a standard (EMAG C4-C22, Supelco® 37 Component Fatty Acid Methyl Esters Mix).

Physicochemical properties

Iodine index, saponification index, peroxide index and titratable acidity were determine for the crude and refined oil, following the methodology described by the AOCS (1997).

β-carotene content and antioxidant capacity

The β-carotene content was determined with the methodology proposed by Barros, Ferreira, Queirós Ferreira & Baptista (2007). 100 µL of oil were diluted with 10 mL of a mixture of acetone: hexane (4:6). The absorbance (A) of the samples was measured at 453, 505, and 663 nm using a spectrophotometer (Genesys 10S UV-VIS, Thermo Scientific, USA). The β-carotene content was calculated using Equation 3.

$$\text{β-carotene (mg of β-carotene/ 100 g of oil)} = 0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453} \qquad (3)$$

The antioxidant capacity was determined using the methodology described by Brand-Williams, Cuvelier & Berset (1995). The oil was diluted with 1 mL of a mixture of ethanol: hexane (1:1), followed by the addition of 0.5 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.3 mol/L. Finally, these samples were left to stand in the dark for 15 min. The absorbance was measured at 540 nm using a spectrophotometer (Genesys 10S UV-VIS, Thermo Scientific, USA).

Oil density and viscosity

The density (ρ) of the crude and refined oils was determined by weighing 50 mL of the oil at 20 °C. The viscosity was determined utilizing a viscometer (RST CC, Brookfield Engineering Labs Inc., USA), using a concentric cylinder geometry at ambient temperature (Sánchez-Machado *et al*., 2015).

Results and discussions

Seed oil extraction

The total oil content in *M. oleifera* seeds was 36.52%. The extraction yield obtained by mechanical pressing was 61% with respect to the oil content of the seed determined by Soxhlet method; this result is in accordance with the value reported by Lalas & Tsaknis (2002) who obtained 60.6% of oil through this extraction method. Mechanical pressing was selected as the oil extraction method because it avoids the use

of organic solvents, which are difficult to remove from the extracted oil (Ruttarattanamongkol, Siebenhandl, Schreiner & Petrasch, 2014).

Oil refining process

The oil recovery yield for each of the refining process stages is shown in Table I. At the end of this process, the recovery yield was 54.66%, which implies a total loss of 45.33%. Degumming, neutralization, and bleaching were the refining stages with the highest oil losses.

During the first stage of the refining process, the degumming stage, hydratable and non-hydratable phospholipids are eliminated from the oil. The former are eliminated by sedimentation or centrifugation of the heated mixture of oil with water. To remove the non-hydratable phospholipids from the oil, acid must be added, which could sometimes forms salts with the metallic complex present in these types of compounds, thus, increasing its solubility in water and its removal.

The elimination of phospholipids improves the sensory quality and hydrolytic stability of the oil. The percentage of oil loss during the degumming stage was 18.66% and probably corresponds to the phospholipid content and to a portion of the oil that remains bound to these compounds (Gupta, 2017).

During the neutralization stage, free fatty acids are eliminated from the oil. These compounds are precursors of oxidation reactions; therefore, their elimination increases the oxidative stability of the oil. Free fatty acids react with the sodium hydroxide added, forming soap, which is separated from the oil by centrifugation and during the washing stage, after the neutralization (Gupta, 2017). The percentage of oil loss during the neutralization stage was 11.66% and corresponds to the free fatty acids content.

Table I. *M. oleifera* oil recovery yield during the refining process.

Refining process stage	Oil recovery yield (%)
Crude oil	100
Degumming	81.34±2.20
Neutralization	69.74±2.05
Washing	66.67±2.56
Bleaching	54.66±2.54

Values represent the average of three repetitions ± standard deviation.

During the bleaching stage, part of the natural pigments of the oil is eliminated because they are trapped in the activated carbon that is removed from the oil (Gupta, 2017). Some of the pigments present in the oil, such as carotenes, act as natural antioxidants and as bioactive compounds; therefore, their elimination can reduce the oxidative stability and the nutritional quality of *M. oleifera* oil (Sánchez-Machado *et al*., 2015). The percentage of oil loss during the bleaching stage was 12.01%.

Acute toxicity of *M. oleifera* seed oil

After the single oral administration of the crude and refined *M. oleifera* oil (300 and 2,000 mg/kg), the animals showed no lethal effect of mortality during the trial period (Table II). Their behaviour and morphological characteristics remained normal. Animals showed no tremors, convulsions, salivation, diarrhoea, and/or lethargy; and no significant differences were observed in their weight (Figure 1).

Table II. Toxicity signals and mortality for the acute toxicity study of crude and refined *M. oleifera* oil.

Group	Dose (mg/kg)	Type of oil	Toxicity signals (TS/NB)[a]	Mortality (D/A)[a]
A	2,000	Refined	0/3	0/3
B	2,000	Crude	0/3	0/3
C	300	Crude	0/3	0/3

[a] Values expressed as number of animals. TS: toxicity signals, NB: normal behavior, D: dead, A: alive.

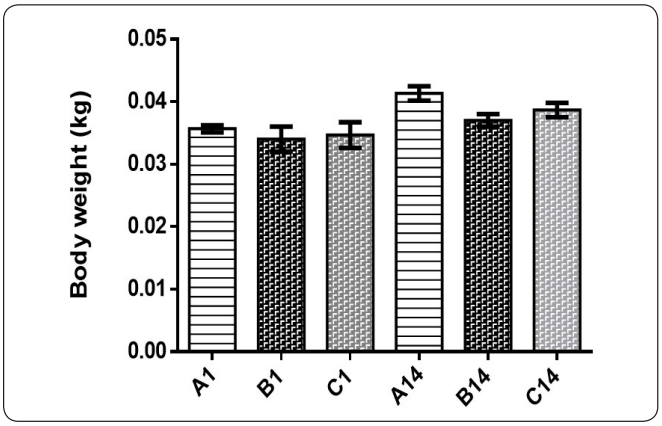

Figure 1. Body weight of mice at the beginning (day 1) and end of the trial (day 14), A: refined oil (2,000 mg/kg), B: crude oil (2,000 mg/kg body weight), and C: crude oil (300 mg/kg body weight).

At the time of the sacrifice, no anatomical changes were observed in the organs of the animals (heart, liver, kidneys, and spleen). Additionally, there was no significant difference in the relative weight of the organs between the different study groups (Figure 2). Due to these results and according to the OECD No. 423 (2001), the *M. oleifera* oil can be considered a safe compound for human consumption.

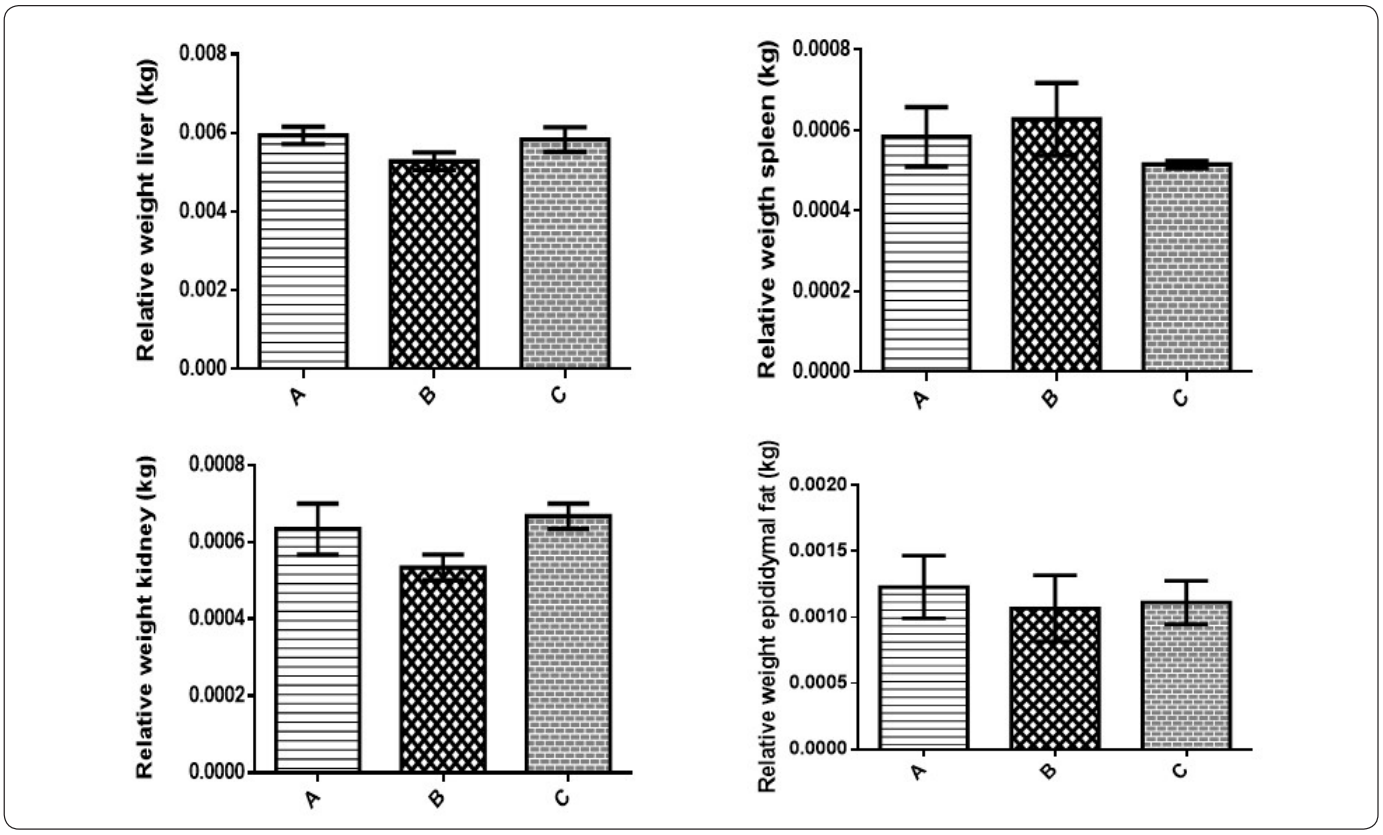

Figure 2. Relative weight of the mice organs in each administration group, A: refined oil (2,000 mg/kg), B: crude oil (2,000 mg/kg), and C: crude oil (300 mg/kg).

The acute toxicity was determined because some previous studies (Al-Anizi *et al*., 2014; Chivapat *et al.,* 2012) reported that the *M. oleifera* seeds present toxicity, which could be associated to the presence of non-lipid compounds (alkaloids, glycosides, and enzymes) associated to oil. In contrast, the results obtained in the present work indicate that the crude and refined *M. oleifera* oil showed no toxicity. This could be attributed to the absence of these compounds in the *M. oleifera* seeds employed for oil extraction. Ukwueze *et al*. (2019) has been informed that the presence of compounds as alkaloids depends on the weather conditions and the geographical location of growth of the plant.

The results obtained in this work suggested that the oil obtained from *M. oleifera* seeds from Mexico, is safe for human consumption. In addition to toxicity, the composition and physicochemical properties of the oil are important to be considered before to use as edible vegetable oil.

Fatty acids profile

The fatty acids profile of the crude and refined *M. oleifera* oil is shown in Table III. The refining process did not cause significant changes ($p < 0.05$) in the fatty acids content of the oil. Sánchez-Machado *et al*., (2015) obtained similar results when evaluating the effect of the refining process on the quality of this oil.

The content of saturated fatty acids of the crude (26.62%) and refined (26.30%) *M. oleifera* oil is appropriate according to the recommendations of the FAO and the European Cardiology Society, who establish that the percentage of these fatty acids in edible oils should not exceed 33% (Eilander, Harika & Zock, 2015).

Oleic acid is the fatty acid present in greater proportion (69%) in *M. oleifera* seed oil; this percentage is similar to the values reported by several authors (Anwar *et al*., 2006; Bhutada, Jadhav, Pinjari, Nemade & Jain, 2016; Sánchez-Machado *et al*., 2015; Sulaiman, Ahmad, Mariod, Mathäus & Salaheldeen, 2017). Since the oleic acid content in *M. oleifera* oil is greater than 60%, it can be classified as a highly monounsaturated vegetable oil (Chiou & Kalogeropoulos, 2017).

In the same classification are olive (78-83% of oleic acid) (Rodrigues *et al*., 2018) and avocado oil (57-60% of oleic acid) (Rodríguez-Carpena, Morcuende & Estévez, 2012). The consumption of olive oil is related to the reduction of total cholesterol and low-density lipoproteins (LDL) in

Table III. Fatty acids profile (g /100 g of fatty acids) of the crude and refined *M. oleifera* oil.

Fatty acid	Crude oil	Refined oil
Saturated (SFA)	26.615±0.124[a]	26.295±0.461[a]
Myristic acid (14:0)	0.097 ± 0.009[a]	0.089 ± 0.003[a]
Palmitic acid (16:0)	5.968 ± 0.061[a]	5.936 ± 0.066[a]
Stearic acid (18:0)	7.510 ± 0.020[a]	7.477 ± 0.139[a]
Arachidic acid (20:0)	4.624 ± 0.007[a]	4.573 ± 0.051[a]
Behenic acid (22:0)	7.399 ± 0.021[a]	7.240 ± 0.160[a]
Lignoceric acid (24:0)	1.017 ± 0.006[a]	0.980 ± 0.042[a]
Monounsaturated (MUFA)	70.595±0.08[a]	70.965±0.243[a]
Cis-Palmitoleic acid (16:1)	1.754 ± 0.006[a]	1.781 ± 0.026[a]
Oleic acid (*cis*) (18:1)	68.841 ± 0.074[a]	69.184 ± 0.217[a]
Polyunsaturated (PUFA)	2.705±0.012[a]	2.701±0.022[a]
Linoleic acid (*cis*) (18:2)	0.735 ± 0.005[a]	0.747 ± 0.006[a]
α-Linolenic Acid (18:3)	1.970 ± 0.007[a]	1.954 ± 0.016[a]

Values represent the average of three repetitions ± standard deviation. Same letters indicate no significant difference between the columns (t-test, $p < 0.05$).

blood, therefore, the consumption of vegetables oils with high percentages of this fatty acid is recommended (Mensink, Zock, Kester & Katan, 2003).

In addition to the oleic acid content, from a nutritional point of view, it is also important to consider the quantity and ratio of linoleic acid (ω6) and α-linolenic acid (ω3) present in the oil. The consumption of these two fatty acids is important because they cannot be synthesized by the organism and because from them, other fatty acids can be synthesized (arachidonic acid, from linoleic; eicosapentaenoic and docosahexaenoic acids from α-linolenic), which develop specific functions in the maintenance of the homeostasis. In order for these fatty acids to generate positive effects on health, they must be consumed in a ω6:ω3 ratio in the range of 1:1 - 2:1 (Saini & Keum, 2018). The analyzed *M. oleifera* oil contains a ω6:ω3 ratio of 0.4:1; thus, the quantity of linoleic acid present in this oil is lower than the recommended quantity (Saini & Keum, 2018). However, in most highly monounsaturated vegetable oils, the quantity of ω6 fatty acids is quite higher than ω3 fatty acids. For example, in olive oil the ratio is 15:1 (ω6:ω3) (Rodrigues *et al*., 2018) and in avocado oil the ratio is 10:1(ω6:ω3) (Rodríguez-Carpena *et al*., 2012). Therefore, the combined consumption of *M. oleifera* oil with olive oil and/or avocado oil could help to reach the recommended fatty acids ratio ω6:ω3.

The plasma cholesterol concentration has been reported to increase if oils with a combined myristic and palmitic acid content of 25% are consumed (Zock, De Vries & Katan, 1994). The combined content of these fatty acids in the crude and refined *M. oleifera* oil is of ~7% in both cases. Therefore, the consumption of the crude or refined *M. oleifera* oil does not increase the plasmatic cholesterol concentration. Another of the saturated fatty acids present in the *M. oleifera* oil is the behenic acid, which is poorly absorbed in the organism (between 11-24%) due to the length of its hydrocarbon chain; thus, its consumption has no significant effect on the plasmatic cholesterol concentration (Cater & Denke, 2001).

Physicochemical characteristics of the oil Iodine value

The iodine value (Table IV) of crude *M. oleifera* oil is similar to the value reported by Anwar *et al*., (2006) of 66.54 g I/100 g of oil; Sánchez-Machado *et al*., (2015) of 63.9 g I / 100 g of oil; and Leone *et al*., (2016) of 65.86 g I/100 g of oil. The refining process increases the iodine value (Table IV). This increase could be due to the loss of some compounds during the refining process, such as phospholipids, which interfere in the binding of iodine with the double bonds of the fatty acids.

Table IV. Physicochemical characterization of the crude and refined *M. oleifera* oil.

Assay	Crude oil	Refined oil
Iodine value (g I/100 g of oil)	65.11± 0.45[a]	70.88 ± 0.54[b]
Saponification value (mg KOH/g of oil)	194.46 ± 3.2[a]	238.46 ± 4.2[b]
Free fatty acids (% oleic acid)	1.87 ± 0.002[a]	0.37 ± 0.0001[b]
Peroxide value (meq/kg)	0.91 ± 0.001[a]	0.65 ± 0.002[b]
β- carotene (mg of β- carotene/ kg of oil)	2.18 ± 0.22[a]	0.66 ± 0.02[b]
Antioxidant capacity DPPH (% of inhibition)	37.24 ± 1.94[a]	15.16 ± 0.47[b]
Density (g/mL)	0.9060 ± 0.003[a]	0.9061± 0.002 [a]
Viscosity (mPa.s)	70 ± 2.3[a]	69 ± 2.7[a]

Values represent the average of three repetitions ± standard deviation. Results with a different letter for each assay are significantly different ($p < 0.05$).

Vegetable oils can be classified in three categories, based on the iodine value. Non-drying oils have an iodine value <100, semi-drying oils between 100 and 140, and drying oils >140 (Gupta, 2017). Therefore, based on this classification,

the crude and refined *M. oleifera* oil is non-drying. In these types of oils, the unsaturated fatty acid content is not enough to form impermeable films in the intestines, which prevent the absorption of nutrients. Such effect is only observed in drying oils, hence, non-drying oils are recommended for human consumption.

Saponification value

The saponification value (Table IV) of the crude *M. oleifera* oil is similar to the value reported for the same oil by Anwar *et al*., (2006) (179-199 mg KOH/g of oil) and by Leone *et al*., (2016) (178-188 mg de KOH /g of oil). After the refining process, the saponification value increased ~23% (Table III), which can be attributed to the removal of compounds with high molecular weight during the degumming stage, specifically phospholipids.

β-carotene content and antioxidant capacity

The β-carotene content (Table IV) of crude *M. oleifera* oil is lower than the value reported by Boukandoul, Casal, Cruz, Pinho & Zaidi (2017), 3.7 ± 0.1 mg of β-carotene/kg of oil. This difference may be due to the variety and to the environmental conditions of the plant development in each case. β-carotene content decreased approximately 70% (Table IV) as a result of the refining process, which can be attributed to the conditions used during such process. During the degumming and neutralization stages, the increase of the oil temperature and the modification of its pH caused oxidation of the carotenes; hence, decreasing the concentration of these compounds in the oil. Additionally, during the bleaching stage, the carotenes are absorbed in the added activated carbon, thus, separating from the oil (Gupta, 2017). Carotenes are pigments that protect oils against oxidation; therefore, the loss of these compounds could increase the degree of oil oxidation (Boukandoul *et al*., 2017).

The antioxidant capacity of the crude and refined *M. oleifera* oil was evaluated by the inhibition of the DPPH radical and expressed as percentage of inhibition. The result obtained for the crude oil (Table IV) is similar to the value reported by Ogbunugafor *et al*., (2011), 48.18 ± 0.01%. The antioxidant capacity of the oil decreased with the refining process (Table IV); this could be due to the reduction of antioxidant compounds content, such as carotenes, tocopherols, and sterols, mainly during the degumming, neutralization, and bleaching stages (Anwar *et al*., 2006).

Oil density and viscosity

The density of the crude and refined *M. oleifera* oil was 0.906 g/mL in both cases, similar to the values reported by Anwar *et al*. (2006) and Sánchez-Machado *et al*., (2015). The density of vegetable oils depends on the degree of unsaturation of the fatty acids that comprise them. Since the fatty acids composition of the oil was not modified during the refining process, the density remained constant.

Viscosity is the measure of the resistance of oils to flow; therefore, it must be considered for the design of processing and transport equipment. It also influences the sensory attributes of the oil, such as palatability. This parameter increases with increasing molecular weight and decreases as the degree of unsaturation of fatty acids present in the oil increases. The viscosity of the crude oil (Table IV) is within the limit values (43-103 mPa.s) reported by Leone *et al*. (2016) for *M. oleifera* oil extracted by cold pressing. The viscosity of the refined oil (Table IV) showed no significant difference ($p < 0.05$) from the viscosity of the crude oil because during the refining process, neither the degree of unsaturation nor the molecular weight of the fatty acids present in the oil were modified.

Free fatty acids (% oleic acid)

The free fatty acids content in the crude *M. oleifera* oil (Table IV) was similar to the value reported by Leone *et al*. (2016) for the same oil, 1-3.5%. The free fatty acids content of the refined oil decreased 80%, due to the reduction of these fatty acids during the neutralization stage. Sánchez-Machado *et al*. (2015) also reported an acidity reduction of *M. oleifera* oil as a result of the refining process, indicating that this reduction increases the oxidative stability of the oil. The acidity percentages obtained in this work for the crude and refined *M. oleifera* oil (Table III) are lower than the maximum limit allowed by the FAO/WHO, 2015 4% and 0.6%, respectively. Considering this standard, the analyzed oils can be used for human consumption.

Peroxide value

The peroxide value obtained for the crude *M. oleifera* oil (Table III) is within the range reported by Anwar *et al.* (2006) and Sánchez-Machado *et al*. (2015), 0.81-1.83 meq/kg. The peroxide value of the refined oil decreased because during the neutralization stage, free fatty acids, which are precursors of these compounds, are removed (Sánchez-Machado *et al*., 2015). The peroxide values obtained for the crude and refined *M. oleifera* oil were lower than the maximum limit allowed (15 meq/kg), established by standard for edible fats and oils not regulated by individual standards. Thus, it can be used for human consumption.

A similar composition is observed when comparing the values of the free fatty acids content and physicochemical characteristics of the analyzed *M. oleifera* oil to the values reported for the olive oil (Allalout *et al*., 2009). Olive oil has a higher polyunsaturated fatty acids content; therefore, this oil presents higher iodine and peroxide values than the analyzed *M. oleifera* oil. Based on these results, *M. oleifera*

oil could be used as an alternative of consumption for highly monounsaturated vegetable oils.

Conclusions

The characterization of the crude and refined oil extracted from the *M. oleifera* seeds from Mexico did not present acute toxicity in a murine model, however, clinical studies are suggested to confirm the security of oil human consumption.

The crude and refined *M. oleifera* seed oil showed that due to their oleic acid content, both could be used as an alternative for the consumption of highly monounsaturated vegetable oils. The content and ratio ω6:ω3 of fatty acids in the *M. oleifera* seed oil could also contribute to reaching the recommended consumption ratio of these fatty acids, generating positive effects on human health. Even though the refining process did not modify the fatty acids content of the oil, it induced the loss of carotenes and other compounds with antioxidant capacity. The oil loss percentage during this process was 45.33%. Therefore, the use of crude oil is suggested, or that during refining, the bleaching process is not carried out, thus avoiding the loss of compounds with antioxidant activity, which allow greater stability to the oil.

The methodology used can be considered useful for the quality control of this natural oil; although, of course, more analysis are necessary.

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