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Biological and toxicological evaluation of edible *Jatropha curcas* L. oil

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Abstract

The non-toxic *Jatropha curcas* flour has been characterized in numerous studies that have demonstrated its nutritional properties and its safe consumption for humans; however, the refined oil has not been characterized biochemically and toxicologically. Its main physicochemical parameters have been determined as well as its fatty acids profile, which showed to be like commercial oils. Its acute toxicity assessment revealed that at a dose of 2000-5000 mg/kg of body weight, the fatty acids were innocuous, indicating its edible grade. Its biological assessment was also performed in rats, using a standard diet, and substituting it with refined and not refined *J. curcas* oil, without observing differences in weight gain, interference in protein efficiency, and change in liver weight due to the oil consumption, indicating that it is safe as food. Hence, the non-toxic *J. curcas* seeds of Mexico could be a source of edible oil.

Keywords: edible oil; *Jatropha curcas*; refined oil; toxicology.

Practical Application: With these studies, the use of non-toxic *J. curcas* oil as edible, for human and animal consumption, is confirmed, making it another food alternative.

1 Introduction

There is a large demand for vegetable sources of edible oils and proteins worldwide. In many countries, including Mexico, large amounts of protein sources and edible oils are imported). Despite programs to foster cultivation of oilseeds crops, there is still a production deficit in Mexico. Currently, soy and palm oil are the main sources of oil; palm oil has been pointed out as non-sustainable due to the deforestation practices that accompany its cultivation (Heilmayr et al., 2020; Ngatirah et al., 2022). The Mexican *piñon* or *xuta* (*Jatropha curcas* L., commonly known as *Jatropha*) is a perennial plant of the *Euphorbiaceae* family. It is native to Central America, but its center of origin is Mexico (Vandepitte et al., 2019). It is widely cultivated in tropical and subtropical regions, but it can survive in extreme climates and soils, not suitable for the development of any other crop. It has been used for reforestation and degraded soil rehabilitation, as it exerts a good control of erosion and phytoremediation (Álvarez-Mateos et al., 2019).

J. curcas has been known to be toxic for a long time, due to the presence of phorbol esters (Makkar et al., 1997), therefore, it is used to produce biofuels or for medicinal and pharmacological purposes, and as life fences by the local populations in many countries (Goel et al., 2007). Consumption of the seeds containing phorbol esters induces nausea, dizziness, and diarrhea; hence all *J. curcas*-derived products are not advisable for human or animal consumption. Phorbol esters are very resistant to heat, which restricts the use of all its products (Makkar et al., 1997).

Notwithstanding, *J. curcas* varieties, identified as nontoxic exist in Mexico, these varieties do not have phorbol esters and, thus, are edible (Vandepitte et al., 2019). These seeds are found in the North of the state of Veracruz, Mexico, and have been widely used for a long time to prepare food for human consumption (Valdes-Rodriguez et al., 2013).

Recently, oil of *J. curcas* seeds has gained interest as a source of renewable energy. The seeds have a high content of oil (43-59%) (Makkar et al., 1997; Martínez-Herrera et al., 2006; Colmenero & Bonilla, 2013) and can contain up to 60% of fatty acids like those of vegetal oils like soy, canola, sunflower, predominating unsaturated fatty acids, like oleic acid (34.3- 45.8%) and linoleic acid (21.51-46.72%) (Colmenero & Bonilla, 2013). Additionally, the amino acids profile and the physicochemical characterization of the oil have been determined and compared with other vegetal sources, like soy, sesame, and sunflower (Carvalho et al., 2019; Kitts et al., 2019). The seed contains high quality oil and proteins (Makkar et al., 1997). Several studies have strengthened the potential of *J. curcas* to be used for human nutrition (Senger et al., 2017; Chino et al., 2019; García et al., 2020).

Vegetal oils can be divided by categories, depending on their processing, in virgin or refined. During the refining process,

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several steps are performed to eliminate unwanted compounds (gums, waxes, etc) and contaminants present, while controlling the formation of new undesirable compounds (Evrard et al., 2007). After these steps, the oil is ready for consumption; hence, the objective of this work is to assess the impact of the refining process on the physicochemical and biological characteristics of the non-toxic *J. curcas* oil for its possible use in the food industry.

2 Materials and methods

2.1 Materials

Edible seeds were harvested from *J. curcas* plantations in the municipality of *Pueblillo*, state of Veracruz, Mexico. The oil from the non-toxic *J. curcas* seeds was extracted with a press (Mod. 510, Korea South) at 450 lb of pressure during 10 min and heating at 85 °C. The obtained oil was divided in two batches to obtain virgin and refined oils.

2.2 Oil refining process

The refining process was performed in four stages: delecithining, degumming, neutralizing, and bleaching-deodorizing. Delecithining consisted in heating the oil to 80 °C and adding water (97:3 v/v oil:water), stirring vigorously during 15 min; then, cooling to 50 °C and centrifuging at 15,680 *g*/15 min. Then, the degumming process followed for which the oil was kept under agitation at 80 °C, adding concentrated HCl (96:4 v/v oil: HCl), softly agitating for 15 min, cooling to 50 °C, and centrifuging at 15,680 *g*/15 min. The next step corresponded to neutralizing, for this the oil was heated to 60 °C, adding 1 N NaOH (85:15 v/v oil: NaOH) under moderate agitation for 15 min, thereafter, increasing the temperature to 75 °C and stirring for 15 min; saturated saline solution (NaCl) was added in a proportion of 90:10 v/v oil: NaCl, temperature was increased to 90 °C, maintaining it for 15 min under agitation. The mixture was cooled to 50 °C, centrifuged at 15,680 *g*/15 min, washed with hot water (90:10 v/v oil: water, 90 °C), and kept under agitation for 15 min. The mixture was cooled and centrifuged at 15,680 *g*/15 min; the latter procedure was performed three times. Finally, the bleaching-deodorizing process was performed, for this, the oil was heated at 90 °C, adding activated carbon (97:3 v/p oil: carbon), keeping it under gentle agitation for 10 min, cooling to 50 °C, and centrifuging (15,680 *g*/25 min). The recovered oil was stored hermetically in an amber flask and refrigerated (4 °C) until its later analysis.

2.3 Physicochemical analysis of J. curcas oil

Crude and refined oils were analyzed by determining color (Lovibond scale), the refractive index in an Abbe refractometer (Atago WA, USA). Following the methods reported by the AOAC (Association of Official Analytical Chemists, 2005), we determined the saponification index (Method 920.160), acidity (Method 940.28), iodine index (Method 920.159), and peroxide index (Method 965.33). The index of esters was determined by calculating the saponification index minus the acidity index.

2.4 Fatty acids profile

Jatropha oil (100 mg) was dissolved in 1 mL of hexane (HPLC, KARAL), adding 100 µL of 5 N sodium methoxide (Sigma-Aldrich, St. Louis, MO, US). After 5 min incubation at 50 °C, 5 mL of distilled water and 0.1 mL of glacial acetic acid (Karal) were added and gently agitated. Fatty acid methyl esters were extracted twice with 3 mL hexane, dried with anhydrous sodium sulfate, and placed in tightly closed vials (Aquino-Bolaños et al., 2017).

FAME analysis was carried out by gas chromatography in a 7090 A GC (Agilent Technologies, Santa Clara, CA, US) coupled to an A5955C mass selective detector (Agilent Technologies). Compounds were separated in an HP-88 column (100 m × 0.250 mm × 0.20 µm, Agilent). The oven program started at 50 °C and was increased to 85 °C at 2.5 °C/min, followed by an increase to 170 °C at 10 °C/min, and kept for 20 min. Finally, the temperature was increased to 250 °C at 10 °C/min, and kept for 25 min. Injection volume was 1 µL at a split ratio of 2:1. Helium was used as carrier gas at 1 mL/min flow. The injection port and interface temperature were established at 250 °C. The total ion chromatograms (TIC) and mass spectra were acquired using an electron impact system (EI) with 70 eV and 1.6 scans/s; the acquisition mass range (m/z) was 30-350. Identification was carried out with the MSD ChemStation E.02.00.493 software (Agilent Technologies) and the National Institute of Standards and Technology (NIST) database, identifying the main compounds. Compounds identity was confirmed by comparing with reference standards and/or by comparison with the Kovats index. Quantification was done with a calibration curve of reference standards of saturated, monounsaturated, polyunsaturated methyl esters of fatty acids (Sigma-Aldrich) (Aquino-Bolaños et al., 2017).

2.5 Assessment of the acute toxicity of the virgin and refined J. curcas oils

The acute toxicity of the virgin and refined oils was assessed following the OECD's Guideline for Testing of Chemicals. Acute Oral Toxicity – Acute Toxic Class Method, Num 423 (Organisation for Economic Co-operation and Development, 2002), the observed signs were alterations in the skin, hair, eyes, mucous membranes, somatomotor activity, and behavioral patterns, aside from the presence of possible tumors, convulsions, salivation, diarrhea, lethargy, and sleepiness.

Four groups of three ICR male mice (PROPECUA S.A. DE C.V.), weighing 25 to 28 g were used. The tested doses were 5, 50, 300, and 2000 mg/kg body weight, applying a single dose in a volume of 1 mL/100 g body weight, using corn oil as vehicle. Administration was done at 8:00 am after fasting using an intragastric cannula for mice. Afterwards, mice received purified water and Rodent Laboratory Chow 5001 (LabDiet, US) *ad libitum*.

2.6 Biological evaluation

To evaluate the effect of Jatropha oil on the development and protein quality of standard food, casein was used as protein source 17 and Jatropha oil (virgin and refined) as a test agent. This evaluation was performed in 36 Wistar rats (18 males and

18 females), recently weaned. Weight gain, daily feed intake, feed conversion, and liver weight were evaluated as an indicator of liver damage. The protein efficiency ratio (PER) was calculated by feeding the rats with the test diets for 28 days and measuring food intake daily and weight gain weekly. Animals were randomly distributed into 4 groups, where the difference in weight per group was not greater than 1 g. Animals were placed in individual cages provided with stainless steel feeders with tight-fitting lids that minimize feed spillage. Water and food were provided *ad libitum*. The experiment was carried out in housing facilities with controlled conditions (21 °C, 55% relative humidity (RH), with a 12/12 h light-dark cycle), according to the guidelines of the institutional Bio-ethics committee (Escuela Nacional de Ciencias Biologicas/IPN) with authorization code CEIENCB-ZOO-009-2019. Food consumption and body weight of the animals were recorded weekly. At the end of the experiment, the animals were euthanized by cervical dislocation, the liver was removed, and the weight was recorded. The applied diet consisted of 15% protein (casein), 10% lipids (corn oil, virgin, and refined Jatropha oil), 4% fiber (non-nutritious cellulose), 1% vitamin (AIN-93-VX), and 4% mineral (AIN-936-MX) mixes that were obtained from Harland Teckland Laboratory Animal Diets (Madison, WI, US) and 66% of carbohydrates (corn flour). The test groups used (n = 9) were: 1) Fat free (adjusting to 81% of carbohydrates), 2) control group (corn oil), 3) virgin jatropha oil group, 4) refined jatropha oil group.

2.7 Statistical analysis

A completely experimental design was used, and all chemical determinations were performed at least in triplicate. All results were reported as means ± SD, and data were compared using GraphPad Prism (R) software version 5.01. ANOVA and parametric tests (Newman-Keuls tests) were performed ($P \leq 0.05$).

3 Results

3.1 Extraction and evaluation of physicochemical properties of J. curcas oil

Table 1 depicts the chemical characterization of the refined and virgin Jatropha oil; these results are particularly interesting because this would be the first study reporting such parameters. Although numerous studies were performed on the Jatropha toxic oil, they have not reached any conclusions about these parameters. On the other side, it can be observed (Table 2) that the values obtained for the saponification, acidity, and esters indexes of the virgin Jatropha oil are within the intervals found in other Jatropha oils sourced from different countries (Islam et al., 2013; Lizarde et al., 2015; Herrera et al., 2019b).

3.2 Acidity index

Due to the refining process, the acidity index of the refined edible *J. curcas* oil diminished to values similar to those reported for other edible oils like that of corn and close to canola (Table 1); the acidity of the virgin oil is within the intervals reported for other *J. curcas* (1.7-10.1) oils sourced from different regions of Mexico and other countries (Table 2). Oils with higher free fatty acid contents possess poor quality, and significant losses occur during the refining process. Therefore, low free fatty acids in crude oil are a physicochemical indicator of quality and would make the refining process unnecessary (Martínez-Herrera et al., 2006; Lizarde et al., 2015).

3.3 Saponification index

The saponification index also increased significantly ($p < 0.05$) in the refined oil as compared to the virgin oil, notwithstanding both values, are within the range reported by other authors in virgin oil of *J. curcas* seeds, which goes from 185 to 198 mg KOH/g) (Islam et al., 2013; Lizarde et al., 2015; Herrera et al., 2019a, b), and within the interval reported by the Mexican standard NMX-F-590-SCFI-2009, for *J. curcas* oil (180-210 mg KOH/g), as shown in Table 1 and for other edible oils.

3.4 Esters index

This value is very close in virgin and refined oils (192.53 and 193.12); this index is determined by calculating the saponification index minus the acidity index. Hence, it will definitively depend on how high the acidity index of the oil is. The soil type also influences high values of the acidity index, and because the soils in La Chontalpa Tabasqueña are acid, the obtained values were very high, 10.1 mg KOH/g. It is known that the esters index

Table 1. Physicochemical characteristics of the virgin and refined *J. curcas* oil.

	virgin oil	refined oil	jatropha oil[5]	corn oil[6]	canola oil[7]
acidity index[1]	0.55 ± 0.00[a]	0.34 ± 0.01[b]	8 max	0.5 max	0.20
saponification index[1]	193.3 ± 2.9[a]	197.9 ± 0.4[b]	180-210	187-195	189
iodine index[2]	90.0 ± 0.7[b]	92.8 ± 0.19[a]	90-110	107-135	108-117
peroxide index[3]	3.44 ± 0.23[b]	2.11 ± 0.59[a]	-	2 max	2.185
esters index	117.21 ± 4.92[a]	111.74 ± 2.39[a]	-	-	ND
specific mass	0.8459 ± 0.003[a]	0.8223 ± 0.004[b]	0.916 max	0.917-0.925	0.917
refraction index	1.468 ± 0.001[a]	1.469 ± 0.001[a]	1.471 max	1.465-1.468	1.465-1.472
physical state (25 °C)	liquid	liquid		liquid	liquid
color[4]	Y = 0.7, B = 0.0, R = 0.0	Y = 0.1, B = 0.0, R = 0.0	-	Y = 35, B = 0.0 R = 4.0	Y = 32 B = -0.0 R = 1.9

[1]mg KOH/g. [2]cg I_2/g. [3]meq O_2/kg. [4]Lovibond scale. Results represent the mean of three assays ± standard deviation (SD). [5]Comité Técnico de Normalización Nacional de la Industria de Aceites y Grasas Comestibles y Similares, 2009. [6]Comité Técnico de Normalización Nacional de la Industria de Aceites y Grasas Comestibles y Similares, 2018. [7]Morales et al., 2006.

allows reaching a better approximation of the average molecular weight of the triglycerides of a fat. Very few publications report the esters index. Therefore, Table 2 was elaborated, in which the esters index of *J. curcas* oils was calculated; no differences are observed among values, including seeds from Mexico and other countries.

It is important to point out that the physicochemical properties of the refined *J. curcas* oil fulfills some characteristics reported for different edible oils, like soy, corn, canola, and jatropha (Comité Técnico de Normalización Nacional de la Industria de Aceites y Grasas Comestibles y Similares, 2018, 2009); hence, its use in the food industry is feasible.

3.5 Iodine index

Iodine index was a significant increased ($p < 0.05$) after the refining process (Table 1), although these values are low as compared with other *J. curcas* virgin oils, both edible and toxic (Lizarde et al., 2015; Herrera et al., 2019b); they are also low as compared to corn and canola oils. However, they are like olive oil, which had a low iodine value (80.3) (Konuskan et al., 2019) indicating that it could be used for human diets. Vegetable oils can be classified into three categories, based on the iodine value. Non-drying oils that have an iodine value < 100, semi-drying oils between 100 and 140, and drying oils > 140 (Gupta, 2017). In this case, the Jatropha oil presented a value < 100, which indicates that it is a non-drying oil. Oils that have higher iodine values (about 190) are used in the paint and varnish industries (Konuskan et al., 2019).

The iodine index is a measure of the degree of unsaturation of oils (Konuskan et al., 2019). It is a constant value for oil, but it depends on the extraction technique used. When the iodine value is low (26-48 g/100 g), the oil is saturated and tends to solidify, whereas, for high values (94-135 g/100 g), the level of unsaturation increases; therefore, the oil remains in a liquid state at lower temperatures and has a lower viscosity (Lizarde et al., 2015). Under this criterion, the virgin and refined oils extracted by the pressing method are prone to solidification at relatively high temperatures. In other countries, such as Costa Rica and Malaysia, iodine indices ranged from 77.14 to 103.62 (Islam et al., 2013; Lizarde et al., 2015).

3.6 Peroxide index

This value diminished in the Jatropha oil after the refining process, from 3.44 to 2.11 mEq O_2/kg (Table 1). The peroxide value is the most widely used. It gives a measure of the extent to which an oil sample has undergone primary oxidation and is determined by measuring the amount of iodine formed by the reaction of peroxides, turned into oil upon primary oxidation with the iodide ion. The oils with peroxide values higher than 2 mEq O_2/kg are prone to rancidity (Islam et al., 2013), so the Jatropha oil does not present this disadvantage. These peroxidation levels are within the range that has been reported for different samples from Mexico and other countries (Islam et al., 2013; Lizarde et al., 2015; Herrera et al., 2019a).

Fresh vegetable oils normally have peroxide values below 10 mEq O_2/kg. High temperature, visible light, and oxygen can easily increase the peroxide value of oils. Only cooking oils with the lowest initial peroxide value are suitable for consumption. Oils with a peroxide value higher than 9 mEq O_2/kg cause undesirable health problems by increasing reactive oxygen species and secondary products of lipid peroxidation that stimulate cardiovascular and inflammatory diseases (Lobo et al., 2010; Lužaić et al., 2022). Therefore, oils with high peroxide values should not be produced and must enforce some regulations to sell highly oxidized cooking oils. Generally, oils with peroxide levels higher than 10 mEq O_2/kg are less stable and have a short shelf-life (Lobo et al., 2010).

3.7 Refraction, specific density, and color indices

The average refraction index of the Jatropha oil from the *Pueblillo*, Veracruz, genotype was 1.46 (Table 1). The refraction index is related to the saturation degree of the oil and indicates the presence of long-chain unsaturated fatty acids. Several authors (Lizarde et al., 2015; Herrera et al., 2019b) reported refraction indices of 1.46 and 1.47 in samples of Jatropha oil from different regions of Mexico.

Specific density values of the virgin and refined oil were very close; however, compared with other values of Jatropha oil, they were below the values that the oil should have, that is, from 0.90 to 0.93 (Islam et al., 2013; Lizarde et al., 2015; Herrera et al., 2019b; Gupta, 2017).

In terms of color, virgin oil has a value of Y = 0.7. As seen clearly in Figure 1, the color is a more intense yellow than that of the refined oil, Y = 0.1, which is much lighter, straw (yellowish); the color of the final oil is essential from the point of view of the consumer, who is used to see an edible oil of a light-yellow color.

Table 2. Comparison of some chemical characteristics of different *J. curcas* oils.

sample	saponification index	acidity index	esters index	reference
virgin oil	197.56	0.55	192.52	
refined oil	193.26	0.34	193.12	
Huimanguillo, Tab., Mex.	195.78	10.09	185.69	Herrera et al., 2019b
Cunduacán, Tab. Mex.	195.70	9.85	185.85	Herrera et al., 2019b
Yautepec, Mor. Mex	187.00	1.68	185.32	Herrera et al., 2019b
Indian	192.34	0.959	191.381	Islam et al., 2013
Indonesia	196.63	1.906	194.724	Islam et al., 2013
South Africa	193.36	0.428	192.932	Islam et al., 2013

3.8 Fatty acids profile

Regarding the fatty acids profile (Table 3) between the virgin and the refined oil, no changes in the fatty acids concentrations were observed; in both oils, a higher than 80% of unsaturated fatty acids was found, which is higher than that reported for other edible oils like olive, sunflower, and canola (Morales et al., 2006; Botero et al., 2014). The content of saturated fatty acids of the virgin oil (15.18%) and refined (16.19%) *Jatropha* oil is appropriate according to the recommendations of the FAO and the European Cardiology Society, who establish that should not exceed 33% (Eilander et al., 2015).

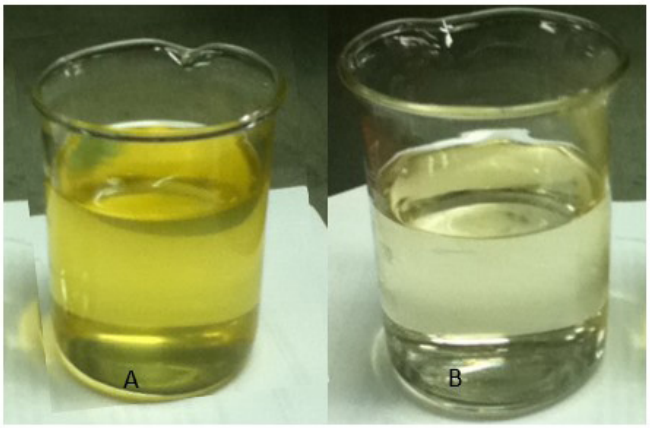

Figure 1. Color of the edible *Jatropha* oil. (A) Virgin oil and (B) refined oil.

In general, the composition of both *Jatropha* oils is like that of corn oil (Høstmark & Haug, 2013), standing out the oleic (> 38%) and linolenic (> 31%) acids content. The *Jatropha* oils, both virgin and refined, presented a lower content of oleic acid (39.02 and 38.37 respectively), and a higher content of palmitic acid: 14.4 and 15.09% respectively, as compared to canola oil: 64-77.6 and 4.2-6.4 respectively (Morales et al., 2006). Oils are one of the main sources of lipids in the human diet and depending on their fatty acids profile they will impact health. Consumption of oleic acid increases the concentration of high-density lipoproteins (HDL) and diminishes the concentration of low-density lipoproteins (LDL) (Torres-Morales et al., 2010; Borges et al., 2022).

Table 3. Fatty acids profile of the virgin (VJO) and refined (RJO) *Jatropha* oil (g/100 g of oil).

fatty acids		VJO	RJO
Myristic acid	C14:0	0.25 ± 0.00[a]	0.27 ± 0.00[a]
Palmitic acid	C16:0	14.40 ± 0.26[a]	15.09 ± 0.54[a]
(Z)-7-Hexadecenoic acid	C:16:0	0.04 ± 0.00[a]	0.05 ± 0.00[a]
Palmitoleic acid	C16:1	0.55 ± 0.00[a]	0.60 ± 0.03[a]
Margaric acid	C:18	0.06 ± 0.00[a]	0.07 ± 0.00[a]
Methyl 8-(2-hexylcyclopropyl) octanoate	C:20	0.02 ± 0.00[a]	0.03 ± 0.00[a]
Stearic acid	C18:1	10.85 ± 0.45[a]	11.56 ± 0.48[a]
NI		0.02 ± 0.00[a]	0.02 ± 0.00[a]
Oleic acid	C18:1n9c	39.02 ± 0.33[a]	38.37 ± 0.50[a]
Olaidic acid	C:181n9t	1.42 ± 0.02[a]	1.48 ± 0.00[a]
NI		----	0.01 ± 0.00
NI		----	0.01 ± 0.00
Linolelaidic Acid	C18:2n6t	0.04 ± 0.00[a]	0.05 ± 0.00[a]
Linoleic acid	C18:2n6c	32.10 ± 0.41[a]	31.02 ± 0.68[a]
NI		0.04 ± 0.01[a]	0.05 ± 0.01[a]
Arachidic acid	C20:0	0.42 ± 0.00[a]	0.45 ± 0.00[a]
Linolenic acid	C18:3n3	0.36 ± 0.01[a]	0.37 ± 0.00[a]
Methyl eicosenoate		0.03 ± 0.00[a]	0.04 ± 0.00[a]
Cis-11-Eicosenoic acid	C21:1	0.13 ± 0.00[a]	0.16 ± 0.00[a]
Metyl octadecadienoate	C21:2	----	0.02 ± 0.00
Heneicosanoic Acid	C21:0	0.01 ± 0.00[a]	0.02 ± 0.00[a]
Behenic acid	C22:0	0.05 ± 0.00[a]	0.06 ± 0.00[a]
Tricosanoic acid	C23:0	0.02 ± 0.00[a]	0.02 ± 0.00[a]
Lignoceric acid	C24:0	0.07 ± 0.00[a]	0.08 ± 0.00[a]
Pentacosanoic acid	C25:0	0.03 ± 0.00[a]	0.03 ± 0.00[a]
Oxiraneoctanoic acid	C19:0	0.02 ± 0.00[a]	0.02 ± 0.00[a]
Hexacosanoic acid	C26:0	0.02 ± 0.00[a]	0.03 ± 0.00[a]
NI		0.02 ± 0.00[a]	0.02 ± 0.00[a]
NI		0.02 ± 0.00[a]	0.02 ± 0.00[a]
Saturated		15.18 ± 0.29[a]	16.19 ± 0.61[a]
Unsaturated		84.46 ± 0.31[a]	83.62 ± 0.59[a]

VJO = Virgin jatropha oil; RJO = Refined jatropha oil; NI = Not identified.

Table 4. Toxicity evaluation of virgin and refined *J. curcas* oils.

product	sex of the animal	risk category	LD_{50} (mg/kg of body weight)
VJO	Male	GHS 5	> 2000
VJO	Female	GHS 5	2000-5000
RJO	Male	GHS 5	2000-5000
RJO	Female	GHS 5	2000-5000

GHS = Globally Harmonized Classification System; VJO = Virgin jatropha oil; RJO = Refined jatropha oil.

Table 5. Biological analysis of the virgin and refined *J. curcas* oils.

Group	AWG	DFC	FC	PER	relative liver weight (%)
fat-free diet	2.34 ± 0.14^{b}	12.03 ± 0.39^{a}	5.25 ± 0.28^{a}	1.94 ± 0.09^{c}	7.18 ± 0.54^{ab}
corn oil, commercial	2.60 ± 0.15^{a}	12.41 ± 0.43^{a}	4.83 ± 0.16^{b}	2.09 ± 0.06^{b}	7.57 ± 0.35^{a}
virgin jatropha oil	2.77 ± 0.16^{a}	12.20 ± 0.39^{a}	4.45 ± 0.11^{c}	2.26 ± 0.06^{a}	6.90 ± 0.34^{b}
refined jatropha oil	2.74 ± 0.11^{a}	12.33 ± 0.23^{a}	4.55 ± 0.19^{c}	2.23 ± 0.09^{a}	6.83 ± 0.39^{b}

AWG = Average weight gain; DFC = Daily food consumption; FC = Feed conversion; PER= Protein efficiency ratio. Values in the same row followed by different superscripts letters mean significant differences ($p \leq 0.05$).

3.9 Toxicological analysis

The virgin and refined *J. curcas* oils (Table 4) can be classified within the GHS 5 category, which implies that they are considered substances with a relatively low toxicity risk. These substances are considered to have an oral LD50 (median lethal dose, 50%) value in the range of 2000 to 5000 mg/kg or < 2000 mg/kg. It is important to point out that no deaths due to the intoxication of animals occurred in any of the toxicity tests, nor were there any clinical signs of toxicity, envisaging lethargy, dullness, and disinclined move, and blood-stained diarrhea. In studies performed with oil from toxic *J. curcas* seeds (Gandhi et al., 1995), with a phorbol esters concentration of 2.5%, the acute oral LD50 of the oil was found to be 6 mL/kg body weight in rats; the oil caused severe diarrhea and gastrointestinal inflammation. Toxic effects have also been seen in goats following oral administration of Jatropha seeds (Gadir et al., 2003) at a dose of 1 and 0.25 g/kg per day. These effects were not present in oils analyzed here.

3.10 Biological assessment

None of the test groups revealed a significant difference in the weight gain, daily food consumption, or the weight of the liver (Table 5). The latter parameter is relevant because the relative weight gain of some organs is due to possible physiological changes that induce an overload of the function of some target organs and inflammatory processes (Alves et al., 2019). Besides the virgin and refined *J. curcas* oils significantly reduced the feed conversion of proteins as compared to the commercial corn oil, without a significant difference between them; it should be noted that in the three diets supplemented with the oil, the feed conversion (FC) diminished significantly respect to the fat-free diet. In PER, the opposite effect was observed, the highest values were obtained in the diets that included *J. curcas* oil, whereas the lowest PER was observed in the fat-free diet.

Consumption of a diet supplemented with *J. curcas* oil did not affect the relative weight of the liver of rats as compared to the fat-free diet; showing a slight diminution ($p < 0.05$) when compared to the diet supplement with corn oil. The latter indicates that *J. curcas* oil can be incorporated safely into feed without affecting PER negatively. Other authors have reported that the weight gain, feed conversion, and the PER increase as the level of digestible energy (lipids) increases in diets for fishes (Gutiérrez et al., 2009). J. *curcas* seeds from nontoxic genotypes are being consumed by human for centuries in the *Totonacapan* region of Mexico.

With these studies, the use of non-toxic *J. curcas* oil as edible, for human and animal consumption, is confirmed, making it another food alternative and the industrial use of edible *Jatropha curcas* oil as an alternative source in the food industry is possible given its physical-chemical characteristics and fatty acid profile.

4 Conclusion

This is the first report that evaluated the refined edible *J. curcas* oil, in terms of its physicochemical properties, fatty acids profile, its toxicity, and its protein efficiency ratio (PER). Based on the results, it can be classified as an innocuous and safe edible oil for human and animal feeding; refining will depend on the quality wished to attain and final use to be determined. Notwithstanding, long-term toxicological studies must be performed to demonstrate that there are no negative effects or damage to any organ, as well as to determine the effect of temperature on processing, storage conditions, among others, and on the different physicochemical parameters of the oil.

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