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Chemical and Physical Characterizaton of *Jatropha curcas* L. Seed from the Northern Sierra of Puebla, México

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Abstractor: *Jatropha curcas* is a valuable plant for its variety of uses. Ten genotypes were collected in the Northern Sierra of Puebla, Mexico, to perform a physical and chemical characterization of the seed. The results indicate that the genotypes San Antonio and La Lagunilla presented the best whole seed size, the highest weights of the kernel and the shell, as the greater length and width of the kernel. Also, stand out in the percentage of kernel and cascara for which they are feasible for its propagation in field. The genotypes Zacapoxtantzingo and Amatlan presented the best characteristics of the proximal analysis so that their seeds can be used for human consumption, while the genotypes Santiago and La Lagunilla present the highest protein content, so it can be considered as food enriching. With respect to the chemical analysis, the 10 genotypes presented six fatty acids: linoleic, oleic, stearic, palmitoleic, palmitic and myristic, of which the oleic and linoleic unsaturated acids are the majority, obtained mainly with the Tuzamapan genotype, while the study of phorbol esters of five of these genotypes indicated that these genotypes showed phorbol esters, but non-toxic genotypes were considered when they presented smaller amounts to the 0.1 mg g^{-1}.

Keywords: *Jatropha Curcas*, Genotype, Physical Characterization, Fatty Acids

1. Introduction

Jatropha, is a genus of the Euphorbiaceae family that has 175 species, 45 of them are in Mexico, where 77% are endemic [1]. In Puebla there are 11 species distributed mainly in the Northern Sierra of Puebla and Mixteca Poblana, all endemic to Mexico, with the exception of *Jatropha curcas*, which reaches Central and South America [2].

In Mexico, *J. curcas* is distributed in 20 states. It is located in tropical and semitropical climates, associated to the low deciduous forest, with precipitations above 600 mm, at altitudes of 0 to 1, 600 meters above sea level, in sandy soils and not suitable for agriculture [3].

Jatropha has a wide variety of uses, including biodiesel production, live fences, erosion control, medicinal products, and cosmetic products such as soap [4]. The seed has high oil content (43 to 59%), according to [5] the cake can contain up to 60% of fatty acids, in patterns similar to edible oils [6]. Unsaturated fatty acids predominate, mainly oleic acid (34.3% to 45.8%) and linoleic acid (29.0% to 44.2%) [7]. Some genotypes are considered to be toxic, since after their oil extraction, when consumed by rats and mice causes poisonings and in humans there are reports of even death [8]. In addition, their seeds contain diterpenes, known as phorbol esters, which cause a purgative effect and some other symptoms such as vomiting, laxative and diarrhea [5]. *J. curcas* has high protein content (22.2% at 28.5) [7] [9].

In addition, only non-toxic materials have been found in Mexico, whose seeds are consumed directly after roasting or in the preparation of traditional dishes by the inhabitants of the Papantla region, Veracruz [10]. In addition, protein utilization in rats and rapid growth in fish suggest a high quality of the protein [11]. This plant in Mexico has great potential, because while the extraction of the oil from its toxic and non-toxic seeds can be used as a substitute for biodiesel, its protein with previous degreasing and warming treatment can be used as a rich ingredient for birds, Livestock pigs and even fish [12].

According to the London-based Global Exchange for Social Investment (GEXSI), the state of Puebla is one of the greatest potential for the cultivation of *J. curcas* (mainly in the Mixteca Poblana) [13], however in the Mixteca Poblana There are no records of non-toxic variants of *J. curcas*, so it is essential to perform the chemical characterization (proximal analysis and fatty acid profile) and physical (length, thickness and width) of edible seeds of the Northern Sierra of Puebla place where people usually consume it) to determine their potential use (industrial and food).

2. Methods

2.1. Seed Collection and Agroclimatic Conditions

Seeds of mature fruits of *J. curcas* were collected in ten localities of five municipalities of the Northern Sierra of Puebla in Mexico (Cuetzalan, Zoquiapan, Ayotoxco de Guerrero, Tuzamapan de Galeana and Xochitlan) in August of 2015. All materials were identified by Dr. Gabriel Flores Franco Herbarium specialist (HUMO) of the Center for Research in Biodiversity and Conservation of the Autonomous University of the State of Morelos. Concerning (1) Cuetzalan municipality, with four localities: Xiloxochitl, Tacuapan, Santiago Yancuitlalpan and Yohualichan (humid semi-calyx region) With rainfall all year), located between LN 20° 04' and 20° 05', LO 97° 28' and 97° 29', 306-518 m altitude, 3759 mm annual precipitation; (2) Zoquiapan municipality with the locality Amatlan (hot humid region with rains all year round), LN 20° 07', LO 97° 28', 518 m altitude, 3588 mm annual precipitation, soil type: cambisol, (3) Ayotoxco municipality of Guerrero with two localities: San Antonio Metzonapa and La Lagunilla; Climate similar to (2); Located between LN 20° 01'and 20° 03', LO 97° 22', 389 472 m altitude, 2090 mm annual precipitation, soil type: vertisol (clayey, expandable and of good fertility), (4) municipality Of Tuzamapan of Galeana with two localities: Tuzamapan of Galeana and El Tuti, climate similar to (2); Located between LN 20° 04'and 20° 06', LO 97° 35'and 97° 32', 447 and 187 m altitude, 2588 annual precipitation, soil type: cambisol, and (5) Xochitlan municipality with The locality Zacapoxtantzingo; Climate similar to (1), LN 19° 57', LO 97° 38', 998 m altitude, 1233 mm annual precipitation, soil type: cambisol (Table 1) [14].

After harvesting, seeds were harvested from the mature fruits of the 10 genotypes which were dried before storage at room temperature (30°C) for 24 h later. Seeds that were empty, deformed and / or colored differently from black were removed. The selected seeds were placed in black plastic bags to be stored at room temperature (about 30°C) for further analysis.

2.2. Physical Characterization of the Seed

Ten seeds were randomly taken from 1 kg of seeds from each locality. The length, width and thickness in cm were estimated using a Surtek® digital calibrator with resolution of 0.01 mm; the weight of the whole seed in g was also recorded with an Ohaus® scale of 2610 g ± 0.1 g. The seed was carefully extracted from the seeds with mechanical forceps and the length and width (cm) and weight (g) of kernel were recorded as well as the weight of the shell, which was calculated from the difference in weight of whole seeds less the weight of the seeds alone.

Table 1. *Geographic and climatic information of Jatropha curcas L. collections in the Northern Sierra of Puebla, Mexico.*

Genotype	Municipality	Location	Altitude (msnm)	Latitude	Longitude	Climate	Soil
1	Cuetzalan	Xiloxochitl	306	20° 04´ 52.0”	97° 28´ 04.3”	ACf	Cambisol y Regosol
2		Tacuapan	316	20° 05´ 2.72”	97° 28´26.6”		
3		Santiago Yancuitlalpan	646	20° 04´ 08.2”	97° 28´ 49.0”		
4		Yohualichan	519	20° 04´ 04.6	97° 29´ 58.5”		
5	Zoquiapan	Amatlán	200	20° 07´ 23.8”	97° 28´ 16.8”	Af	Cambisol
6	Ayotoxco de Guerrero	San Antonio Metzonapa	389	20° 03´ 06.2”	97° 22´ 46.0”	Af	Vertisol
7		La Lagunilla	472	20° 01´ 22.1”	97° 22´ 18.1”		
8	Tuzamapan de Galeana	Tuzamapan de Galeana	447	20° 04´ 16.9”	97° 35´ 06.2”	Af	Cambisol
9		El Tuti	187	20° 06´ 20.4”	97° 32´ 22.6”		
10	Xochitlan	Zacapoxtantzingo	998	19° 57´ 58.5”	97° 38´ 45.7”	ACf	Cambisol

ACf= Wet semi-warm with rain all year round. Af= Warm humid with rain all year round.

2.3. Proximal Composition

For this the kernel without shell was used, which was crushed, using a mechanical mill to obtain the kernel flour, from which dry matter, crude protein, lipids and ash content were determined. The dry matter was determined by placing in the trays one gram of sample of ground kernel seed in an oven at 105°C for 4 hours. Moisture was determined by difference of total weight and dry matter. Raw protein, lipids and ash contents were determined according to the methodology proposed by the AOAC (1990) [15]. The protein was obtained by the Kjedahl method, for the digestion a Buchi digestion unit K-424 was used and for distillation the Buchi Distillation Unit K-350. Lipid extraction was carried out using petroleum ether and a Soxhlet Buchi (E-812) fat extraction equipment. In the ash determination, the flour samples were previously calcined, and then a four hour at 550°C was introduced into the muffle (Thermolyne 6000). Carbohydrates (nitrogen-free extract) were determined by difference in the sample of protein, ash and fat percent.

2.4. Analysis of Fatty Acid Composition

The seed oil was extracted using petroleum ether, at a temperature of 70°C, at a ratio of 1:26 (w/v) for 2.5 h (13 recirculations of the solvent on average). Preparations of methyl esters were made from total lipids by the AOAC method (1990). The methyl esters were analyzed by an Agilent Technologies (7890A GC System) gas chromatograph coupled to an Agilent Technologies Mass Spectrometer (5975C V2-MSD with Triple-Axis Detector), with a self-injector and injector (G4513A) and a spectroscopy detector of masses. The temperature gradient range in the column was 60 to 255°C; as carrier gas helium was used with a flow range of 1.39 ml per minute. A mixture of fatty acid standards was injected and the retention times were used to identify the peak samples. Fatty acid levels were estimated as percentage of total area of the peak of methyl esters.

2.5. Extraction and Estimation of the Phorbolesters by HPLC

The phorbol esters were determined of the genotypes 1 (Xiloxochitl), 2 (Tacuapan), 3 (Santiago), 6 (San Antonio) and 7 (La Lagunilla). About 2 g of defatted kernels from each genotype were weighed and subsequently extracted with HPLC grade methanol and methanol as described by Makkar *et al.* (1998a b) [10] [16]. The analytical column was RP-18 reverse phase (Lichrospher 100.250 x 4 mm, RP 18, 5 µm) Agilent 280 x 20 mm ID The separation was performed at room temperature (30°C) and the flow rate was 1 ml min^{-1}. The five peaks of phorbol esters that appeared between 10 and 12.5 min were identified and integrated at 280 nm. The results are expressed as equivalent to standard phorbol-12-myristate 13-acetate, which appeared at 11.098 min.

3. Results and Discussion

3.1. Physical Characterization of Seed

The physical characteristics of the 10 genotypes of *J. curcas* are shown in Table 2. Genotypes 7 (0.87 g, La Lagunilla) and 6 (0.82 g, San Antonio) showed the highest weights of the complete seeds. Genotypes 1 (0.64 g, Xiloxochitl) and 9 (0.65 g, El Tuti) had the lowest weight. The weight of the kernel and the shell was higher again in the genotypes 7 (0.58 and 0.32 g, La Lagunilla) and 6 (0.55 and 0.29 g, San Antonio). Regarding length and width seeds, genotypes 6 (2.06 and 1.05 cm, San Antonio) and 7 (1.97 and 1.08 cm, La Lagunilla) stand out (Table 2).

The values of seed weights of the 10 genotypes were higher than those found by [12], but similar to those reported by [10] [17], and lower than those of [18], in *J. curcas* of Colombia (1.2 g). In addition, the seed length and width of the 10 genotypes were similar to those reported by [19] and higher than those of [12]. As well as kernel weight and shell weight, it was higher than that reported by [12].

Of the kernel length, the genotypes 7 (1.64 cm, La Lagunilla) and 6 (1.61 cm, San Antonio) and 4 (1.56 cm, Yohualichan) stand out, while the kernel width stand out genotypes 3 (0.89 cm, Santiago), 7 (0.87 cm, La Lagunilla) and 4 (0.87 cm, Yohualichan) (Table 2). Regarding the kernel width, results were obtained superior to those found by [12], since these authors registered from 6.1 to 7.7 cm the width of the kernel.

In the highest percentage of kernel and lower percentage of shell, the genotypes 3 (68.52 and 31.48%, Santiago) and 1 (65.59 and 34.41%, Xiloxochitl) were the highest percentage of kernel and lower percentage of the shell, followed by genotypes 6 (64.85 and 35.15%, San Antonio) and 7 (64.74 and 35.26%, La Lagunilla) (Table 2). The percentage results of kernel and shell are similar to those found by [20] [12] [16]. In contrast, [10] obtained lower results in percentage of kernel (60.1 to 63.5%) and higher in percentage of shell (37.3 to 40%), of equal effect [21] recorded a lower percentage of kernel (62 to 62.8%) and a higher percentage of shell (37.2 to 38%) in *J. curcas* of Colombia.

Table 2. *Physical characterization of seed in Jatropha curcas L. collections in the Northern Sierra of Puebla, Mexico.*

	Whole Seed			Kernel			Shell	% of	% of
Genotype	Weight (g)	Length (cm)	Width (cm)	Length (cm)	Width (cm)	Weight (g)	Weight (g)	kernel	shell
1	0.64 d	1.81 c	0.96 b	1.49 bc	0.79 c	0.42 d	0.22 d	65.59 b	34.41 a
2	0.71 bcd	1.86 bc	0.99 ab	1.52 abc	0.82 bc	0.48 bcd	0.26 bc	64.52 b	35.48 a
3	0.76 abc	1.83 c	1.04 ab	1.52 abc	0.89 a	0.55 abc	0.25 bcd	68.52 a	31.48 b
4	0.75 bcd	1.90 bc	1.05 ab	1.56 ab	0.87 ab	0.50 bcd	0.28 bc	64.18 b	35.82 a
5	0.74 bcd	1.94 abc	1.01 ab	1.53 abc	0.82 bc	0.49 bcd	0.27 bc	64.48 b	35.52 a
6	0.82 ab	2.06 a	1.05 ab	1.61 a	0.83 abc	0.53 ab	0.29 ab	64.85 b	35.15 a
7	0.87 a	1.97 ab	1.08 a	1.64 a	0.87 ab	0.58 a	0.32 a	64.74 b	35.26 a
8	0.71 bcd	1.89 bc	0.99 ab	1.53 abc	0.82 bc	0.48 bcd	0.27 bc	63.69 b	36.31 a
9	0.65 cd	1.82 c	0.98 ab	1.43 c	0.79 c	0.43 cd	0.25 cd	63.53 b	36.47 a
10	0.77 ab	1.88 bc	1.03 ab	1.48 bc	0.82 abc	0.48 ab	0.26 bc	64.34 b	35.66 a

1. Xiloxochitl. 2. Tacuapan. 3. Santiago. 4. Yohualichan. 5. Amatlan. 6. San Antonio. 7. La Lagunilla. 8. Tuzamapan. 9. El Tuti. 10. Zacapoxtantzingo. Means followed by the same letter do not differ significantly (Tukey; $p > 0.05$).

These results show that genotypes 6 and 7 (San Antonio and La Lagunilla collected in the municipality of Ayotoxco de Guerrero) presented the best whole seed size, the highest weights of the kernel and the shell, as the greater length and width of the kernel. Also, stand out in the percentage of kernel and shell. These genotypes are feasible to propagate in the field; however, it is necessary to evaluate chemical and organic fertilization, since they can improve the physical and chemical characteristics of the seed and increase yield [22], [23]. Everything is important to increase the weight of kernel and percentage of the same with respect to the whole seed.

3.2. Proximal analysis, Dry Matter and Moisture

Dry matter contents varied from 97.04% in genotype 5 (Amatlan) to 95.50% in genotype 10 (Zacapoxtantzingo). In contrast, genotype 10 (4.5%, Zacapoxtantzingo) recorded the highest moisture content with respect to genotype 5 (2.96%, Amatlan), which obtained the lowest moisture content. The dry matter of the 10 genotypes is similar to those reported by [10] [16] [17]; as equal or superior to [12]; while moisture of the 10 genotypes is similar to [24] [25], and lower than [21] with values greater than 4.5%.

The genotypes 2 (62.39%, Tacuapan), 4 (61.35%, Yohualichan) and 1 (61.33%, Xiloxochitl) of the municipality of Cuetzalan had the highest total fat content, which is similar to that recorded by [26] [10] [16] [17] [24], but higher than that reported by [27] [19], with values between 28.6 and 48.07% fat. A high fat content of *J. curcas* may favor the production of biodiesel [28], such as the production of industrial products such as kerosene, soap, cosmetics, pesticides or lubricants [4].

Of the crude protein, the genotypes 3 (27.38%, Santiago) and 7 (25.73%, La Lagunilla) of the state of Puebla were the most important. This protein content was similar to that reported by [9] [10] [24] [26]; However [12] [16] [17] reported higher protein contents of *J. curcas* from the states of Michoacan, Morelos, Quintana Roo and Veracruz in Mexico (26.1 to 34.5%) and [5] in African countries (19 to 31%). Due to the high protein content in *J. curcas*, it would be feasible to use it as a food enriched based on maize and wheat, p.e. corn tortillas fortified with non-toxic *Jatropha curcas* L. flour increased their protein by 6.20% [29], especially if the seeds do not contain phorbol esters or if they present amounts less than 0.1 mg g^{-1} of these compounds [12]. In addition, jatropha protein has the potential to be used as a paper adhesive, biofilm formulation and as an emulsifier [30], or as a feed for cattle [31].

The genotypes 10 (5.02%, Zacapoxtantzingo) and 9 (4.87%, El Tuti) of the state of Puebla had the highest values of ash, while genotypes 1 and 2 (3.7%, Xiloxochitl and Tacuapan) had the lowest values of ash. In another study, Martínez *et al*. (2006) [12] obtained the highest ash contents with seeds of *J. curcas* of San Isidro (5.1%, Yautepec, Morelos) and Pueblillo (4.7%, Papantla, Veracruz), while seeds of Castillo of Teayo (3.8%) and Coatzacoalcos (3.9%) of Veracruz recorded the lowest ash content.

The highest carbohydrate contents were obtained from genotypes 5 (15.24%, Amatlan), 1 (15.07%, Xiloxochitl) and 10 (14.7%, Zacapoxtantzingo), but these were lower than the carbohydrates of seeds with the *J. curcas* Paraguay and Argentina (38.0 to 44.1%) [27]. Based on the above [17] asserts that non-toxic *J curcas* would improve food security in tropical countries by providing edible oil in roasted seeds and seed press cake as a source of protein for humans and animals.

Table 3. *Proximal seed analysis of Jatropha curcas L. collections in the Northern Sierra of Puebla, Mexico.*

Genotype	Dry Matter (%)	Moisture (%)	Lipid (%)	Protein (%)	Ash (%)	Carbohydrates (%)
1	96.82 ab	3.18 de	61.33 ab	19.81 f	3.79 ef	15.07 a
2	96.58 bcd	3.42 bcd	62.39 a	20.10 f	3.78 f	13.73 bcde
3	96.94 a	3.06 e	55.23 e	27.38 a	4.19 cde	13.20 de
4	96.62 bcd	3.38 bcd	61.35 ab	20.28 f	4.11 def	14.26 abcd
5	97.04 a	2.96 e	57.43 d	24.10 cd	3.82 ef	15.24 a
6	96.36 d	3.64 b	59.12 c	23.18 ed	4.27 cd	13.42 cde
7	96.51 cd	3.49 bc	55.41 e	25.73 b	4.49 bcd	14.37 abc
8	96.34 d	3.66 b	60.24 bc	22.29 e	4.58 bc	12.89 e
9	96.73 abc	3.27 cde	57.01 d	25.43 bc	4.87 ab	12.69 e
10	95.50 e	4.5 a	56.20 ed	24.08 cd	5.02 a	14.70 ab

1. Xiloxochitl. 2. Tacuapan. 3. Santiago. 4. Yohualichan. 5. Amatlan. 6. San Antonio. 7. La Lagunilla. 8. Tuzamapan. 9. El Tuti. 10. Zacapoxtantzingo. Means followed by the same letter do not differ significantly (Tukey; $p > 0.05$).

3.3. Fatty Acid Profile and Phorbol Esters

The fatty acids compositions of the different genotypes of *J. curcas* were recorded in Table 4. The fatty acids found in all genotypes were: linoleic, oleic, stearic, palmitoleic, palmitic and myristic. The main fatty acids present in genotype 8 (Tuzamapan) were oleic; linoleic and stearic while in genotype 10 were palmitic and palmitoleic acids. In addition, myristic fatty acid has high values in genotypes 6 (San Antonio) and 9 (El Tuti). This variation is possible due to the genetics of these genotypes, such as climate variation and soil type [12]. These results show that the oil consists mainly of unsaturated fatty acids (oleic and linoleic). These results are similar to those reported in other mexican states [32] [33] [12] [19], as in others countries [34] [7] [26]. However, there are other studies where seed oil of *J. curcas* presented as the main fatty acid linoleic followed by oleic in Brazil [35] or Egypt [36].

It should be noted that *J. curcas* has a higher content of oleic acid (40.3%) compared to soybean (24%), sunflower (17.7%) and rapeseed (33%) in contrast to linoleic acid (37%), comparison with soybean (54%) and sunflower (72.9%) [37]. In addition, the same author states that the composition and content of esters of fatty acids directly influence the properties of biodiesel, and consequently the quality and its performance as fuel and the fatty acids of the *J. curcas* confer good physicochemical characteristics to biodiesel according to the standard of the American Society for Testing and Materials. It is important to mention that the defatted kernel flour in the five analyzed genotypes presented phorbol esters, but at low levels. These seeds could therefore be used in the food preparation industry. However, it is necessary to test in mice to confirm their safety.

Table 4. *Fatty acid profile (% of area) in seed of Jatropha curcas L. collections in the Northern Sierra of Puebla, Mexico.*

Genotypes	Oleic (C18:1)	Linoleic (C 18:2)	Palmitic (C 16:1)	Stearic (C 18:0)	Palmitic (C 16:0)	Myristic (C 14:0)	Total phorbolesters (mg/g)*
1	32.70 bcd	31.41 cd	1.23 b	5.82 a	15.89 ab	1.63 ab	0.052
2	37.64 abc	27.62 e	2.51 a	5.86 a	13.82 abc	0.92 b	0.049
3	32.16 cd	32.09 cd	1.13 b	5.60 a	13.44 abc	1.53 ab	0.034
4	29.90 d	25.69 e	1.10 b	91 a	12.82 abc	1.29 ab	----
5	38.35 ab	30.94 d	1.41 b	5.69 a	14.38 abc	1.80 ab	----
6	30.39 d	33.78 bc	1.30 b	6.86 a	11.60 c	2.94 a	0.045
7	30.86 d	25.87 e	1.47 b	6.86 a	12.85 abc	1.61 ab	0.054
8	40.85 a	37.61 a	0.89 b	7.37 a	12.36 bc	1.25 ab	----
9	35.07 abcd	35.69 ab	0.87 b	6.42 a	12.41 bc	2.19 ab	----
10	32.32 cd	33.26 bcd	2.56 a	5.97 a	16.13 a	0.98 b	----

1. Xiloxochitl. 2. Tacuapan. 3. Santiago. 4. Yohualichan. 5. Amatlan. 6. San Antonio. 7. La Lagunilla. 8. Tuzamapan. 9. El Tuti. 10. Zacapoxtantzingo. Means followed by the same letter do not differ significantly (Tukey; p> 0.05).
*Equivalent to 12-myristate, 13 acetate

4. Conclusions

The genotypes 6 (San Antonio) and 7 (La Lagunilla) of the municipality of Ayotoxco de Guerrero presented the best physical characteristics of seed, which is why they are feasible candidates to propagate in the field. The genotypes 10 (Zacapoxtantzingo) and 6 (Amatlán) presented the best characteristics of the proximal analysis so that their seeds could be used for human consumption, while the genotypes Santiago and La Lagunilla present the highest protein content and can therefore be considered as enriching food. The unsaturated oleic and linoleic acids are the majority, obtained mainly from genotype 8 (Tuzamapan). The genotypes showed phorbol esters, but non-toxic genotypes were considered when they were less than 0.1 mg g^{-1}.

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